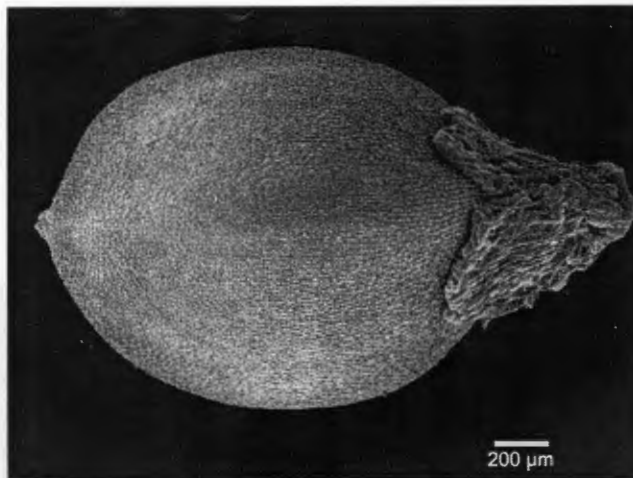




**Phylogenetic Inference and Macro-
evolutionary Patterns in *Ficinia* Schrad.
(Cyperaceae)**



F. stolonifera nutlet

**BY: CAITLYNNE M. FRANCIS (FRNCAI001)
SUPERVISOR: DR A M MUASYA**

**A dissertation submitted to the University of Cape Town in partial fulfillment of
the requirements for the award of an Honours degree in Botany**

2009

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

OCTOBER 2009

Abstract

The genus *Ficinia* Schrad. has its centre of diversity in the Cape Floristic Region and phylogenetic relationships within this genus have not been fully studied representing a gap in the body of work on the *Cyperaceae*. Phylogenetic analyses provide strong support for the sister relationship between *Isolepis* and *Ficinia* in both parsimony and Bayesian analyses (BP = 96; PP = 1). *Isolepis marginata* consistently resolved as more closely related to the *Ficinia* clade than to the rest of the species in *Isolepis*. *Ficinia undosa* was positioned outside its own genus (as well as the entire *Ficinia-Isolepis* clade) rendering the genus paraphyletic. This species appears to be more closely related to members of the *Scirpus falsus-Scoipoides* clade. As in previous studies, the New Zealand monotypic genus *Desmoschoenus* is resolved as a member of *Ficinia* and is closely allied with *Ficinia pallens* (BP=43, PP=0.56). Macro-evolutionary reconstruction of characters such as life form and the presence of a gynophore disk revealed interesting patterns within the genus as well. Perennial habit is a synapomorphy for *Ficinia* and distinguishes it from the generally annual sister-genus *Isolepis*. Though a young genus when compared with *Isolepis*, *Ficinia* has radiated in the CFR quite rapidly following the shift toward Mediterranean climate in this region. While support across the tree is quite low, due perhaps to the slow mutation rate among recent and rapidly radiated perennials, several relationships within in the genus are resolved with moderate nodal support.

are there
nodes
contradicting?

Introduction

Speciation in the Cape Floristic Region (CFR) is a topic that has aroused the interest of many biologists (e.g. Van der Niet & Johnson 2009, Linder and Hardy 2004). This interest is sparked in no small measure by the sheer number of species present in this relatively small (in comparison with other floral kingdoms especially) stretch of land (Kruger & Taylor 1979). Goldblatt and Manning (2000, 2002) report a species count of around 9000 with a staggeringly high percentage (68.7%) of species endemic to the region. The angiosperms comprise about 8 888 of the estimated 9 000 species found in the CFR. This diversity is brought about in part by a range of factors such as the rugged landscape, variable rainfall patterns and a variety of soil types that create a mosaic of distinct habitats in close proximity to each other (Goldblatt and Manning 2002).

The *Cyperaceae* is a well-established member of the monocots and third largest family. Of the 104 genera that make up this family, 30 are monotypic (e.g. *Hellmuthia*) while *Carex* is among the largest genera of angiosperms with about 1700 species (Goetghebeur, 1998 Goldblatt & Manning, 2000). Members of this family are used in traditional medicines (e.g. *Kyllinga triceps* Rottb.) as well as other economic endeavours for example paper production and traditional roofing material in Africa is harvested from various sedges such as *Cyperus papyrus* L. and *C. rotundus* L. respectively (Simpson & Inglis, 2001).

Sedges, as the *Cyperaceae* are more commonly referred to as, are found in a variety of habitats including, but not limited to, granite and limestone outcrops and rock overhangs in the Swartberg and Karoo Mountain regions of the Western Cape. This versatility in habitat and the *Cyperaceae*'s well-documented persistence throughout the African continent are key features noted by Gordon-Gray (1995). Drawing on these features Gordon-Gray (1995) highlights the untapped potential this family represents as a bio-indicator and tracker of climatic change. Several incidences of reclassification and taxonomic revision have occurred in the last century and the classification of the family is as a result quite unstable (Gordon-Gray 1995). That said significant progress has been made in last few years to address this instability and tie together genera into a useful and sound taxonomic account of the family.

Within the Cyperaceae, the genus *Ficinia* Schrader consists of around 60 species most (90%) of which grow in the CFR with a few growing in and around Kwa-Zulu Natal, Eastern Cape as well as tropical Africa and Australasia (Muasya *et al* 2009, Muasya, 2005). Originally described by Schrader, this genus of (mostly) perennial shrubs has a diverse range in habitat and altitude and can be diagnosed by a number of characters. Muasya (2005) summarises these as the presence of a gynophore disk at the base of the nutlet in most species, often exhibiting a papery leaf sheath and ligule as well as bisexual flowers lacking perianth segments.

The gynophore disk is the most prominent character distinguishing *Ficinia* from other genera in the *Ficinia* Clade as described by Muasya *et al* (2009). This 'hypogynous disk' is a fleshy growth at the base of the nutlet (Vrijdaghs *et al* 2005) with a large variation in size and this has been suggested as a character that may further distinguish species within the genus (AM Muasya pers. comm.). However, the boundaries between *Ficinia* and its sister genus *Isolepis* is somewhat blurred by the presence of the gynophore in *Isolepis marginata* (Muasya *et al* 2001); the absence of it in *Ficinia* species such as *F. filliformis* (this taxon has been previously named *F. tenuifolia* until Gordon-Gray (2008) clarified the nomenclature) (Muasya 2005).

While *Isolepis*, a predominantly annual genus (Muasya and Simpson 2002), has been examined to some extent both morphologically and molecularly in terms of phylogenetics (e.g. Muasya *et al* 2001) such works on *Ficinia* are still in progress. The mode of speciation for this African genus is as yet unknown and reviewing literature reveals little to no studies has been done on this matter. This is a considerable gap in the knowledge we have on the sedges and especially so for a genus with its centre of diversity in the Cape Floristic Region.

With this study, we aim to contribute to the volume of work on the genus by focusing in on the following questions:

1. What are the phylogenetic relationships within *Ficinia*?
2. What are the broad-scale patterns of character evolution within *Ficinia* and how do these characters of interest give insight into the biological history of the genus?

Materials and Methods

Molecular Data

A total of 113 specimens (82 species with 31 replicates) formed the core material - both molecular and morphological - upon which this study was based. Total DNA was extracted from silica-dried plant material using the CTAB isolation method (modified from Gawel and Jaret 1991) for 25 species drawn from the collections of Dr AM Muasya (see Table 1). Sequences for the remaining 88 ^{specimens} ~~species~~ are a part of unpublished worked by DR AM Muasya.

One nuclear and two plastids regions were amplified under standard PCR methods. The primers used for the nuclear region were Internal Transcribed Spacer (*ITS1*, 5.8S and *ITS2*) (White *et al* 1991) and the primers for the plastid region were the *rps16* intron (Oxelman *et al* 1997) and *trnL-F* {Taberlet *et al* 1995}. The PCR reactions were performed in 30µl volumes consisting of 18.6µl sterile distilled water, 3µl of 10x DNA polymerase buffer, 3µl of MgCl₂ (50mM), 1µl each of the forward and reverse primers (10µM), 1.2µl of dNTP (10mM), 0.2µl of *Taq* DNA polymerase and 2µl of template DNA. The reaction was carried out on an applied Biosystems GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The profile of *ITS*, *trnL-F* and *rps16* intron had an initial denaturation phase of 2minutes at 94°C, followed by 35 cycles of 60 seconds at 94°C, 60 seconds at 52°C and 2 minutes at 72°C. The final extension phase of 7 minutes was done at 72°C. The PCR products were run on a 1% agarose gel stained with Goldview™ to evaluate the success of the PCR amplification. Successfully amplified PCR products were sent to the DNA sequencing facility at Stellenbosch University, South Africa for sequencing using the respective primers used for amplification. Sequences from this study was appended to an existing matrix

Automatic sequence alignment was done using the ClustalW pairwise alignment in BioEdit with minor adjustments made manually where ClustalW failed to recognize regions of alignment within the matrix.

Morphological Data

A total of eighteen (18) morphological characters were scored in this study (See table 2) and all characters with the exception of Nutlet Surface Morphology and Major Phytogeographical Centres were scored from specimens in the Bolus Herbarium,

Table 1: Taxa analysed in the combined matrix of this study (Unpub = Sequences as yet unpublished by DR AM Muasya; CMF = sequences produced in this study and dashed (-) symbols indicate missing sequences)

Taxon	Voucher Number	ITS	rps16	TrnL-F
<i>Desmoschoenus spiralis</i> Hook.f.	GENT 0699	Unpub	Unpub	-
<i>Ficinia acuminata</i> (Nees) Nees	Muasya 3796	CMF	CMF	CMF
<i>Ficinia aff acuminata</i>	Muasya 3247	CMF	CMF	-
<i>Ficinia albicans</i>	Muasya 2358	Unpub	Unpub	Unpub
<i>Ficinia anceps</i> Nees	Muasya 3817	CMF	CMF	CMF
<i>Ficinia aff anceps</i>	Muasya 2219	Unpub	Unpub	Unpub
<i>Ficinia angustifolia</i> (Schrad.) Levyns	Muasya 2885	Unpub	Unpub	Unpub
<i>Ficinia anysbergensis</i> Muasya	Muasya 3085	Unpub	Unpub	Unpub
<i>Ficinia argyropa</i> Nees	Muasya 2363	Unpub	Unpub	Unpub
<i>Ficinia bergiana</i> Kunth.	Muasya 2233	Unpub	Unpub	Unpub
<i>Ficinia brevifolia</i> Nees ex Kunth.	Muasya 2337	Unpub	Unpub	Unpub
<i>Ficinia bulbosa</i> (L.) Nees	Muasya 2205	Unpub	Unpub	Unpub
<i>Ficinia capillifolia</i> (Schrad.) C.B. Clarke	Muasya 2359	Unpub	Unpub	Unpub
<i>Ficinia capitella</i> (Thunb.) Nees	Muasya 4132	Unpub	Unpub	Unpub
<i>Ficinia cedarbergensis</i> T.H. Arnold & Gordon-Gray	Muasya 2325	Unpub	Unpub	Unpub
<i>Ficinia compasbergensis</i> Muasya	Muasya 2313	Unpub	Unpub	Unpub
<i>Ficinia deusta</i> (P.J. Bergius) Levyns	Clark 171	CMF	CMF	-
<i>Ficinia dunensis</i> Levyns	Muasya 2215	Unpub	Unpub	Unpub
<i>Ficinia eklonea</i> (Steud.) Nees	Muasya 3966	Unpub	Unpub	Unpub
<i>Ficinia eliator</i> Levyns	Muasya 3154	CMF	CMF	CMF
<i>Ficinia esterhuyseniae</i> Muasya	Muasya 2345	Unpub	Unpub	Unpub
	Muasya 2215	Unpub	Unpub	Unpub
	Muasya 2312	Unpub	Unpub	Unpub

Taxon	Voucher Number	ITS	rps16	TrnL-F
<i>Ficinia fascicularis</i> Nees	Muasya 2966	Unpub	Unpub	Unpub
<i>Ficinia fastigiata</i> (Thunb.) nees	Muasya 3825	CMF	CMF	-
	Muasya 2230	Unpub	Unpub	Unpub
	Grimshaw 93939	Unpubl	Unpubl	Unpubl
	Muasya 3755	CMF	CMF	CMF
	Muasya 3226	CMF	CMF	CMF
<i>Ficinia gracilis</i> Schrad.	Muasya 2355	Unpubl	Unpubl	Unpubl
<i>Ficinia grandiflora</i> T.H. Arnold & Gordon-Gray	Muasya 2331	Unpub	Unpub	Unpub
<i>Ficinia gydomonata</i> T.H. Arnold	Muasya 2333	Unpub	Unpub	Unpub
	Muasya 3952	Unpub	Unpub	Unpub
<i>Ficinia indica</i> (Lam.) Pfeiffer	Musaya 2909a	Unpub	Unpub	Unpub
<i>Ficinia aff indica</i>	Muasys 2909b	Unpub	Unpub	Unpub
	Muasya 2301	Unpub	Unpub	Unpub
<i>Ficinia ixiodes</i> Nees	Muasya 2207	Unpub	Unpub	Unpub
<i>Ficinia laciniata</i> (Thunb.) Nees	Muasya 2340	Unpub	Unpub	Unpub
<i>Ficinia lateralis</i> (Vahl) Kunth	Muasya 3863	CMF	CMF	CMF
<i>Ficinia levynsiae</i> T.H. Arnold & Gordon-Gray	Muasya 3956	Unpub	Unpub	Unpub
<i>Ficinia macowanii</i> C.B. Clarke	Muasya 4129	Unpub	Unpub	Unpub
<i>Ficinia micrantha</i> C.B. Clarke	Muasya 2268	Unpub	Unpub	Unpub
<i>Ficinia minutiflora</i> C.B. Clarke	Muasya 2257	Unpub	Unpub	Unpub
<i>Ficinia monticola</i> Kunth	Muasya 2287	Unpub	Unpub	Unpub
<i>Ficinia nigrescens</i> (Schrad.) J. Raynal	Muasya 2197	Unpub	Unpub	Unpub
	Muasya 2350	Unpub	Unpub	Unpub
	Muasya 9455	Unpub	Unpub	Unpub
<i>Ficinia nodosa</i> (Rottb.) Goetgh., Muasya & DA Simpson	Muasya 3299	CMF	CMF	CMF
<i>Ficinia oligantha</i> (Steud.) J. Raynal	Muasya 3094	Unpub	Unpub	Unpub
<i>Ficinia aff oligantha</i>	Muasya 3179	CMF	CMF	CMF

Taxon	Voucher Number	ITS	rps16	TrnL-F
<i>Ficinia pallens</i> (Schrad.) Nees	Muasya 3955	Unpub	Unpub	Unpub
	Muasya 3098	Unpub	Unpub	Unpub
<i>Ficinia paradoxa</i> (Schrad.) Nees <i>Ficina aff paradoxa</i>	Muasya 4164	CMF	CMF	CMF
	Muasya 2338	Unpub	Unpub	Unpub
	Muasya 2300	Unpub	Unpub	Unpub
	Muasya 2364	Unpub	Unpub	Unpub
<i>Ficinia petrophila</i> T.H. Arnold & Gordon-Gray	Muasya 3100	Unpub	Unpub	Unpub
	Muasya 1183	Unpub	Unpub	Unpub
<i>Ficinia pinguor</i> C.B. Clarke	Muasya 2320	Unpub	Unpub	Unpub
	Muasya 2330	Unpub	Unpub	Unpub
<i>Ficinia polystachya</i> Levyns	Muasya 2348	Unpub	Unpub	Unpub
<i>Ficinia praemorsa</i> Nees	Muasya 2296	Unpub	Unpub	Unpub
<i>Ficinia pygmaea</i> Boeck.	Muasya 3804	CMF	CMF	CMF
<i>Ficinia sp nov - limestone</i>	Clark 620	CMF	CMF	-
<i>Ficinia sp-Clark</i>	Muasya 347	-	Unpub	Unpub
<i>Ficinia sp</i>	Muasya 4127	Unpub	Unpub	Unpub
<i>Ficinia quanquangularis</i> Boeck.	Muasya 2310	Unpub	Unpub	Unpub
<i>Ficinia radiata</i> (L. f.) Kunth	Clark 350	CMF	CMF	-
<i>Ficinia ramosissima</i> Kunth	Muasya 2268	Unpub	Unpub	Unpub
<i>Ficinia repens</i> Kunth	Muasya 2347	Unpub	Unpub	Unpub
<i>Ficinia rigida</i> Levyns	Muasya 2319	Unpub	Unpub	Unpub
	Muasya 2211	Unpub	Unpub	Unpub
<i>Ficinia secunda</i> (Vahl) Kunth	Muasya 3968	Unpub	Unpub	Unpub
	Muasya 3771	CMF	CMF	CMF
<i>Ficinia stolonifera</i> Boeck.	Muasya 2715	Unpub	Unpub	Unpub
<i>Ficinia filliformis</i> (Lam.) Schrad.	Muasya 3948	Unpub	Unpub	Unpub

Taxon	Voucher Number	ITS	rps16	TrnL-F
<i>Ficinia trichodes</i> (Schrad.) Benth. & Hook. f.	Muasya 2328	Unpub	Unpub	Unpub
<i>Ficinia trispicata</i> (L. f.) Druce	Muasya 2252	Unpub	Unpub	Unpub
<i>Ficinia tristachya</i> (Rottb.) Nees	Muasya 4141	Unpub	Unpub	Unpub
	Muasya 3797	CMF	CMF	CMF
<i>Ficinia truncata</i> (Thunb.) Schrad.	Muasya 2361	Unpub	Unpub	Unpub
<i>Ficinia undosa</i> B.L Burt	Muasya 3701	CMF	CMF	CMF
<i>Ficinia zeyheri</i> Boeck.	Muasya 4139	Unpub	Unpub	Unpub
<i>Hellmuthia membranacea</i> (Thunb.) R.W. Haines & Lye	Muasya 3081	Unpub	Unpub	Unpub
<i>Isolepis fluitans</i> (L.) R. Br.	Muasya 1007	Unpub	Unpub	Unpub
	Muasya 3135	Unpub	Unpub	Unpub
	Muasya 961	Unpub	Unpub	Unpub
<i>Isolepis bicolor</i> Carmich.	Muasya 105	Unpub	Unpub	Unpub
<i>Isolepis meruensis</i> Lye	Muasya 1061	Unpub	Unpub	Unpub
<i>Isolepis wakefieldiana</i> (S.T. Blake) K.L. Wilson	Muasya 1108	Unpub	Unpub	Unpub
<i>Isolepis costata</i> A. Rich.	Muasya 1109	Unpub	Unpub	Unpub
<i>Isolepis ludwigii</i> (Steud.) Kunth	Muasya 1138	Unpub	Unpub	Unpub
<i>Isolepis diabolica</i> (Steud.) Schrad.	Muasya 17804	Unpub	Unpub	Unpub
<i>Isolepis striata</i> (Nees) Kunth	Muasya 2316	Unpub	Unpub	Unpub
<i>Isolepis digitata</i> Nees ex Schrad.	Muasya 2258	Unpub	Unpub	Unpub
<i>Isolepis rubicunda</i> (Nees) Kunth	Muasya 1154	Unpub	Unpub	Unpub
	Muasya 1221	Unpub	Unpub	Unpub
<i>Isolepis marginata</i> (Thunb.) A. Dietr.	Muasya 17452	Unpub	Unpub	Unpub
	Muasya 2973	Unpub	Unpub	Unpub
	Muasya 2999	Unpub	Unpub	Unpub
<i>Isolepis inyangensis</i> Muasya & Goetgh.	Muasya 2025	Unpub	Unpub	Unpub
<i>Isolepis hystrix</i> (Thunb.) Nees	Muasya 2971	Unpub	Unpub	Unpub

Taxon	Voucher Number	ITS	rps16	TrnL-F
<i>Isolepis montivaga</i> (S.T. Blake) K.L. Wilson	Muasya 9489	Unpub	Unpub	Unpub
<i>Isolepis capensis</i> Muasya	Muasya 3019	Unpub	Unpub	Unpub
	Muasya 3078	Unpub	Unpub	Unpub
<i>Scirpus falsus</i> C. B. Clarke	Muasya 3740	CMF	CMF	CMF
<i>Scirpus ficinoides</i> Kunth	Muasya 8841.14	CMF	CMF	-
<i>Scirpoides dioecus</i> (Kunth) J. Browning	Muasya 2294	Unpub	Unpub	Unpub
<i>Scirpoides holoschoenus</i> (L.) Soják	Muasya 1205-	Unpub	Unpub	Unpub
<i>Scirpoides burkei</i> (C.B. Clarke) Goetgh., Musaya & D.A. Simpson	Muasya 3762	CMF	CMF	CMF
<i>Schoenoplectus leucanthus</i> (Boeck.) J. Raynal	Muasya 2882	Unpub	Unpub	Unpub

UCT. Nutlet Surface Morphology was scored from Scanning Electron Microscope (SEM) photographs provided by DR AM Muasya (See Figure 4) and Major Phytogeographical Centres scored from Goldblatt & Manning's *Cape Plants: A Conspectus of the Cape Flora of South Africa* (2000). Where information for the genus *Isolepis* could not be scored from herbarium specimens, characters were scored from the monograph of *Isolepis* (Muasya and Simpson, 2001).

Data Analysis

The complete matrix consisted of 2809 characters: 2791 molecular characters formed from *rps16* intron (905), *ITS* (766) and *trnL-F* (1120) as well as morphological characters (18). Gaps were coded as missing in the DNA matrix as well as where no specimens were available for morphological character scoring. Dual analyses on a matrix of only molecular characters and a combined morphological-molecular matrix for the 113 taxa in this study was carried out to infer the relationship within *Ficinia* and selected members of its sister genus *Isolepis*. The phylogenetic analysis of relationships between these taxa were performed in PAUP* version 4.0b10 (Swofford, 2002) using the parsimony algorithm and also in MrBayes Version 3.12 (Huelsenbeck & Ronquist, 2003). Parsimony analysis was done under the Fitch criterion of unordered, equal weights (Fitch 1971). Heuristic tree searches were performed with 1000 random replicates and tree bisection-reconnection (TBR) swapping holding only ten (10) trees at each replicate to reduce time spent searching on below-optimum trees (Muasya and Simpson 2001). All trees generated were swapped to completion. Nodal support was evaluated through bootstrap analysis of 1000 replicates (Felsenstein, 1985) with characters sampled using equal weighting (Fitch, 1971). Trees were constructed on the basis of simple taxon addition and TBR branch swapping; groups with frequencies greater than 50% in the final bootstrap consensus tree were retained. Following Muasya *et al* (2001) the following descriptions for categories of bootstrap support will be used: weak, 50-74%; moderate, 75-84%; strong, 85-100%.

Bayesian inference of phylogeny with posterior probabilities (PP) as measures of support was done using Mr Bayes. Only the combined molecular data matrix with was analysed under the GTR+I+ Γ model of molecular evolution for each data set and default MrBayes priors. This model was chosen because the impact of

overparameterization on the accuracy of the model search in the tree space is lower than that of underparameterization (Huelsenbeck & Rannala 2004). A Markov Chain Monte Carlo (MCMC) algorithm was employed in the analyses. Two simultaneous runs were done starting from random trees with four chains (three heated and one cold chain) set and the temperature was raised from the default 0.2 to 0.3 to emulate a previous Bayesian inference done on the tribe *Cypereae* (Dludlu, 2007). The initial analysis ran for 2.5 million generations and the sampling frequency was set at 100 i.e. Markov chains were sampled every 100th generation. Analyses were run until the average standard deviation of the split frequencies approached 0.10, which is indicative of convergence onto a stationary distribution. However, the initial and subsequent analyses failed to converge despite increasing the number of generations to 5 million and the sampling frequency to 1000. The point of stationarity generally provides a guideline for the number of trees to be discarded from the initial search of the model, termed the 'burn-in period'. Because the runs failed to converge a larger burn-in period was advised (Dr. Y Bouchenak-Khelladi pers. comm.). Calculation of posterior probability (PP) was done after discarding trees sampled during this burn-in period.

Macro-evolutionary patterns were analysed by tracing the morphological character evolution using a parsimony reconstruction method in Mesquite version 2.71 (Maddison & Maddison 2009) based on the parsimony tree. *none detected?*

Table 2: Character Scoring in this study

Taxon	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Desmoschoenus_spiralis	1	1	0	1	1	0	2	1	0	-	-	0	-	-	0	0	-	2
I_fluitans1	1	0	1	1	1	0	0	1	1	0	2	0	0	0	0	7	3	2
I_bicolor	0	0	1	1	1	1	1	1	1	0	3	1	1	2	1	0	0	3
I_meruensis	0	0	0	1	1	1	0	1	1	-	4	0	1	4	0	0	3	0
I_wakefeldiana	0	0	0	1	1	1	1	0	1	0	3	1	1	4	0	0	1	2
I_costata	0	1	0	1	1	0	2	1	1	0	3	0	1	3	0	0	1	0
I_ludwigii	1	0	0	1	1	0	0	1	1	0	3	0	1	0	0	7	1	1
I_rubicunda	0	0	0	1	1	1	1	1	1	1	0	0	1	3	0	3	1	0
I_diabolica	1	0	0	1	1	1	1	1	1	0	3	0	2	4	0	6	0	0
I_striata	1	1	1	1	1	0	0	1	1	0	3	1	0	0	0	6	1	0
F_pinguor1	1	1	0	0	1	1	1	1	0	0	-	0	1	0	0	5	1	1
I_rubicunda2	1	0	1	1	1	0	1	1	1	1	0	0	1	0	0	5	0	1
I_digitata	1	0	0	1	1	0	1	1	1	1	0	0	1	0	0	6	1	1
I_marginata	0	0	0	1	1	1	1	1	0	1	4	0	0	0	0	1	1	2
I_inyangensis	0	0	1	1	1	0	0	0	1	0	3	0	1	4	0	0	4	0
F_nigrescens1	1	0	0	1	1	0	2	1	0	0	1	0	1	0	0	0	1	1
F_angustifolia	1	1	1	1	1	0	1	0	0	0	2	1	1	0	0	1	3	1
F_oligantha	1	0	0	1	1	1	1	1	0	0	1	0	1	0	0	1	0	1
F_brevifolia	1	1	0	1	1	1	2	1	0	1	-	0	1	0	0	1	1	1
F_ixiodes	1	1	0	0	1	0	1	1	0	1	4	0	2	0	0	6	3	1
F_zeyheri	1	0	0	1	1	1	0	1	0	0	1	1	1	0	0	7	2	1
F_secundal	1	1	0	1	1	1	1	0	0	1	2	1	1	0	0	1	2	1
F_deusta	1	1	0	0	1	0	2	1	0	0	4	1	2	0	0	1	0	1
F_eliator	1	1	0	1	1	0	1	1	0	0	-	0	1	0	0	5	0	1
F_dunensis1	1	1	0	1	1	0	1	0	0	1	4	0	2	0	0	1	0	1
F_pinguor2	1	1	0	0	1	1	1	1	0	0	-	0	1	0	0	5	1	1
F_anceps	1	0	0	1	1	1	1	1	0	0	-	1	1	0	0	5	1	1
F_stoloniferal	1	0	0	0	1	1	1	1	0	1	1	0	2	0	0	1	1	1

Taxon	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
F_pallens	1	1	0	1	1	0	1	1	0	0	-	0	1	0	0	5	0	1
F_capillifolia	1	0	1	1	1	0	1	0	0	1	-	0	0	0	0	6	0	1
F_fastigiata	1	1	0	1	1	0	1	0	0	1	-	1	1	0	1	5	1	1
F_argyropa	1	0	0	0	1	1	1	1	0	1	2	0	1	0	0	5	0	1
F_macowanii	1	1	0	1	1	0	1	1	0	1	0	0	1	0	0	5	2	1
F_trispicata	1	1	0	1	1	0	1	1	0	1	2	1	1	0	1	7	1	1
F_tristachyal	1	0	0	1	1	0	1	1	0	1	1	0	1	0	0	7	0	0
F_minutiflora	1	0	0	1	0	1	2	1	0	1	-	0	1	0	0	5	1	1
F_levynsiae	1	1	0	1	1	0	1	1	0	1	-	0	2	0	0	5	2	1
F_micrantha	1	0	0	1	1	1	0	1	0	1	2	0	1	0	0	5	1	1
F_monticola	1	1	0	1	1	0	1	1	0	1	3	1	1	0	0	5	2	1
F_amosissimal	1	0	1	1	1	0	1	1	0	1	3	0	1	0	0	7	0	1
F_pygmaea	1	1	0	1	1	0	0	1	0	1	-	0	1	0	0	6	0	1
F_aff_paradoxal	1	1	0	0	1	0	1	1	0	0	1	0	1	0	0	5	0	1
F_aff_indical	1	1	0	1	1	0	2	1	0	0	1	1	1	0	0	0	0	1
F_radiata	1	0	0	1	1	0	1	1	0	1	4	1	0	1	1	7	0	1
F_esterhuyseniae	1	0	0	1	1	0	2	1	0	1	3	1	1	0	0	2	0	0
F_cedarbergensis	1	0	0	0	1	1	2	1	0	0	4	0	1	0	0	2	2	1
F_indical	1	1	0	1	1	0	2	1	0	0	1	1	1	0	0	0	0	1
F_rigida	1	1	0	1	1	0	2	1	0	1	-	0	1	0	0	5	0	1
F_polystachyal	1	1	0	1	1	0	1	1	0	1	1	1	1	0	0	5	2	1
F_capitella	1	0	0	1	1	0	1	0	0	1	1	0	1	0	0	6	2	1
F_trichodes	1	1	1	1	1	1	0	0	0	1	3	1	1	0	1	5	0	1
F_polystachya2	1	1	0	1	1	0	1	1	0	1	1	1	1	0	0	5	2	1
F_grandiflora	1	1	0	1	1	1	2	1	0	0	2	1	1	0	0	5	3	1
F_gydomonata	1	0	0	1	1	0	1	1	0	0	-	0	1	0	0	2	1	1
F_bergiana	1	1	0	1	1	1	1	1	0	0	1	0	1	0	0	5	3	1
F_paradoxa	1	1	0	0	1	0	1	1	0	0	1	0	1	0	0	7	0	1
F_laciniata	1	0	0	1	1	1	1	1	0	1	3	0	1	0	0	7	0	1

[illegible]

Taxon	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
F_gracilis2	0	1	0	1	1	1	2	1	0	1	5	0	1	0	0	4	0	1
F_nodosa1	1	1	0	1	1	1	2	1	0	1	0	0	2	0	0	5	-	2
I_montivaga	0	0	0	1	1	1	1	1	1	0	0	0	1	-	0	0	3	2
I_fluitans3	1	0	1	1	1	1	0	1	1	0	3	0	0	0	0	7	3	2
Scirpoides dioecous	1	1	0	1	1	1	0	2	1	1	0	0	1	0	0	1	0	0
S_holoschoenus	1	1	0	1	1	1	0	2	1	1	0	0	1	0	0	6	0	0
F_compasbergensis	1	1	0	1	1	1	2	1	0	1	-	1	1	1	0	5	3	1
F_amosissima2	1	0	1	1	1	1	0	1	1	0	1	3	0	1	0	7	0	1
F_sp	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F_lateralis1	1	0	0	1	1	1	1	1	0	0	1	0	1	0	0	0	0	1
F_dunensis2	1	1	0	1	1	1	0	1	0	1	4	0	2	0	0	1	0	1
F_sp aff oligantha	1	0	0	1	1	1	1	1	0	0	1	0	1	0	0	1	0	1
F_gracilis3	0	1	0	1	1	1	2	1	0	1	5	0	1	0	0	4	0	1
F_acuminata1	1	1	0	1	1	1	1	0	0	1	2	0	1	0	0	1	2	0
F_nodosa2	1	1	0	1	1	1	2	1	0	1	0	0	2	0	0	5	-	2
F_undosa	1	-	-	-	-	-	-	1	-	-	3	-	-	-	-	-	-	-
Scirpus falsus	1	0	0	1	1	1	1	1	1	0	0	0	1	0	0	1	3	0
F_gracilis4	0	1	0	1	1	1	2	1	0	1	5	0	1	0	0	4	0	1
S_burkei	1	1	0	1	1	1	0	2	1	1	0	0	1	0	0	-	3	0
F_stolonifera3	1	0	0	0	1	1	1	1	0	1	1	0	2	0	0	1	1	1
F_acuminata2	1	1	0	1	1	1	1	0	0	1	2	0	1	0	0	1	2	0
F_tristachya2	1	0	0	1	1	1	0	1	1	0	1	1	1	0	0	7	0	0
F_sp nov	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F_albicans	1	0	0	1	1	1	2	1	0	0	0	0	1	0	0	5	0	1
F_fascicularis	1	1	0	1	1	1	2	1	0	1	2	0	0	0	0	5	0	1
F_lateralis2	1	0	0	1	1	1	1	1	0	0	1	0	1	0	0	0	0	1
S_ficinoides	1	1	0	1	1	1	1	1	1	1	0	0	2	0	0	-	3	0

Table 3: Morphological Characters and symbol information used in Table 2

Morphological Character	Character state and Scoring
A: Life Form	Annual (0); Perennial (1)
B: Plant Size	<30cm (0); >30cm (1)
C: Internodes on Culm	One (0); Multiple (1)
D: Leaf Sheath Appearance	Papery (0); Not papery (1)
E: Leaf Blade Size	<5mm (0); >5mm (1)
F: Position of Inflorescence	Terminal (0); Pseudolateral (1)
G: Number of Spikelets/Inflorescence	1 (0); 2-10 (1); >10 (2) Distichous (0); Spiral (1)
H: Glume Arrangement	Present (0); Absent (1)
I: Gynophore Disk	3 (0); 2 (1)
J: Stigma Branching	Smooth (0); Raised & Star-like (1); Dented (2);
K: Nutlet Surface Morphology (NSM)	Raised & Papillae (3); Raised & Round (4); All States (5)
L: Inflorescence Type	Capitate (0); Spicate (2)
M: Root Structure	Minute (0); short (<5mm) rhizome (1); stolon (2)
N: Substrate	Sandy/Shale (0); Granite/Limestone (1); Atlantic Islands (2); Witteberg Quartzite (3); Other (4)
O: Habitat	Open (0); Closed (1)
P: Phytogeographical Centres	Not Applicable (0); All Centres (1); NW Dominant (2); KM Dominant (3); LB Dominant (4); SW Dominant (5); NW, SW, AP (6); KM, LB, SE (7); AP dominant (8)
Q: Altitude	<300m (0); 300m-900m (1); 900m-1500m (2); >1500m (3)
R: Biomes	Summer Rainfall (0); Winter Rainfall (1); Australasia (2); Atlantic Islands (3)

Results:

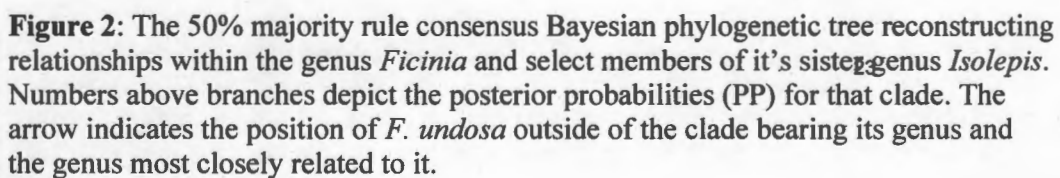
Phylogenetic Analyses

The parsimony analyses performed on the combined matrix had 1973 constant characters, 332 characters are variable but parsimony uninformative, and 504 parsimony informative characters. From the heuristic search, 68 equally parsimonious trees of length 2727 were found, each with a consistency index (CI) of 0.475 and the retention index (RI) of 0.620. One of the trees from this analysis is presented in Figure 1 with branch lengths and bootstrap proportions indicated above and below the branches, respectively. Because the Bayesian analyses failed to converge a larger burn-in was discarded (4000 out 5000 trees) and the posterior probabilities were calculated from the remaining 1000 trees. The 50 % majority consensus tree obtained is presented in Figure 2, showing the posterior probabilities above the branches. In the parsimony analysis *Hellmuthia membranacea*, *Schoenoplectus leucanthus*, *Scirpus falsus*, *Scirpus ficinoides*, *Scirpoides holoschoenus*, *Scirpoides dioecous* and *Scirpoides burkei* formed the group used to root the tree. For the Bayesian analysis a single taxon, *Hellmuthia membranacea*, was used to root the group. Figure's 2 and 3 show a broadly similar topology with the Bayesian tree depicting for more polytomies than the Parsimony analysis. For the description of main features I will refer to Figure 1 – the parsimony tree.

The results of the combined matrix, as shown in Figure 1, show all the taxa belonging to *Ficinia* (with the exception of *F. undosa*) forming moderately supported clade (BP = 72). The nodal support for the sister relationship between the *Ficinia-Isolepis marginata* clade and the remaining *Isolepis* species (in this study referred to as *Isolepis* proper) is strongly supported (BP=96, PP=1) resolving the same relationships as previous studies such as Muasya and Simpson (2002). This node forms the point of shared ancestry between *Isolepis* and *Ficinia*. Surprisingly *F. undosa* is resolved as more closely related to two of the *Scirpoides* species that forms the rooting group in this phylogeny along with three species of the genus *Scirpus*.

Resolution is evident within *Ficinia* but poorly supported at the nodes of the major clades with multiple instances of bootstrap proportions below 50% (not indicated on Figure 1). However, towards the tips of the tree the BP values increase slightly with certain nodes reaching levels of weak to moderate support (BP = 51 (PP=1) for the

both of the
clades are
weakly
supported



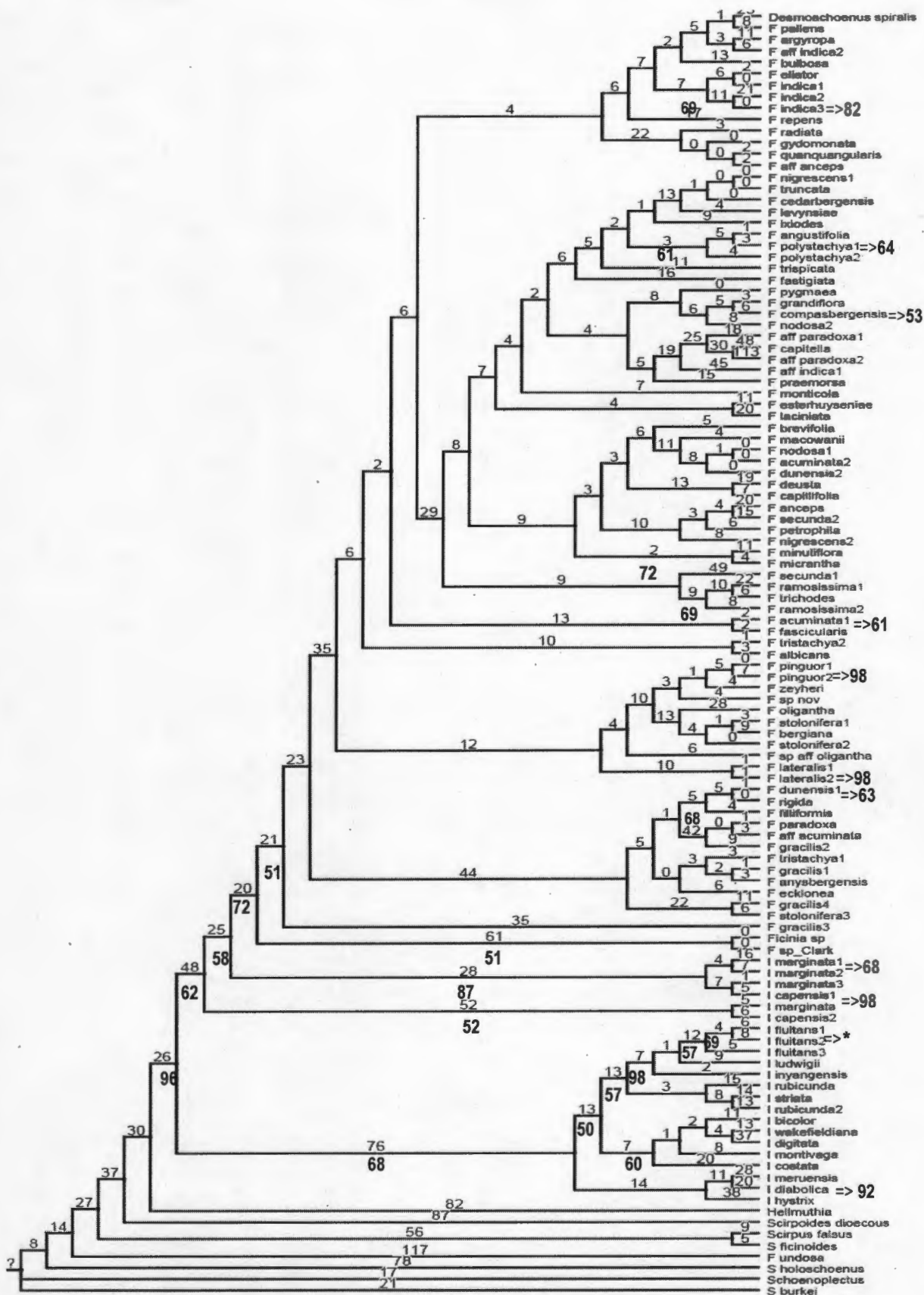


Figure 1: Parsimony reconstruction based on the combined data matrix for *Ficinia* and the closest genera within *Cyperaceae*. Numbers above the branches are branch lengths and numbers below are bootstrap proportions (BP) and * =100 BP. Sister relationships with high BP are indicated next to the relevant species' name.

relationship between *F. sp Clarke* and *Ficinia sp* and the rest of the genus). Several relationships have moderate support; for example the clade consisting of *F. eliator* and *F. indica* has a BP of 69 and the relationship between *F. rigida*, *F. dunensis* and *F. filliformis* also generates support a reasonable level of support in comparison with other parts of the tree (BP = 69). The *Isolepis* 'proper' (i.e. excluding the *I. marginata*-*I. capensis* grouping sister to the *Ficinia* clade) clade has slightly higher bootstrap support (BP = 68) subtending that node with the internal nodes varying from well supported (BP=98) or weakly supported (BP=60).

Of the selected sedges that make up the outgroup in this study, *Hellmuthia membranacea* is more closely related to the *Ficinia-Isolepis*, a relationship that had previously been found in other studies as well.

Morphological Character Reconstruction

Table 2 shows the morphological characters how they varied across the taxa. Ancestral reconstructions for these characters are depicted in Figure 3 a - r. In Figure 3a we see that the perennial life form exhibited by all members of *Ficinia* is the ancestral state with annual life form arising independently and only in the *Isolepis*. Plant size reconstruction (Figure 3b) shows that the ancestral state was one of a smaller (<30cm) size but with several instances of reversals between states occurring across the tree. Having multiple internodes appears to be a derived trait, which has evolved independently at least three times in *Ficinia* and once in the *Isolepis fluitans* clade (Figure 3c). Certain members of *Ficinia*, in particular the clade in which *F. stolonifera* and *F. pinguor* are present, exhibit a papery leaf sheath (Figure 3d).

A single instance of smaller leaf blade size has evolved in *F. minutiflora* (Figure 3e) with all other species both in the *Ficinia-Isolepis* clade and the sedges that form the outgroup retaining an ancestral trait of larger leaf blade size (>5mm). While pseudolateral inflorescence position appears to be the ancestral state a significant number of *Ficinia* - many of whom form a distinct clade - have developed terminal inflorescence positions (Figure 3f).

The retention of larger numbers spikelets per inflorescence from the ancestral state is apparent across the tree with some species even exhibiting the evolution of more than 10 spikelets per inflorescence (Figure 3g). Spiral glume arrangement is the ancestral

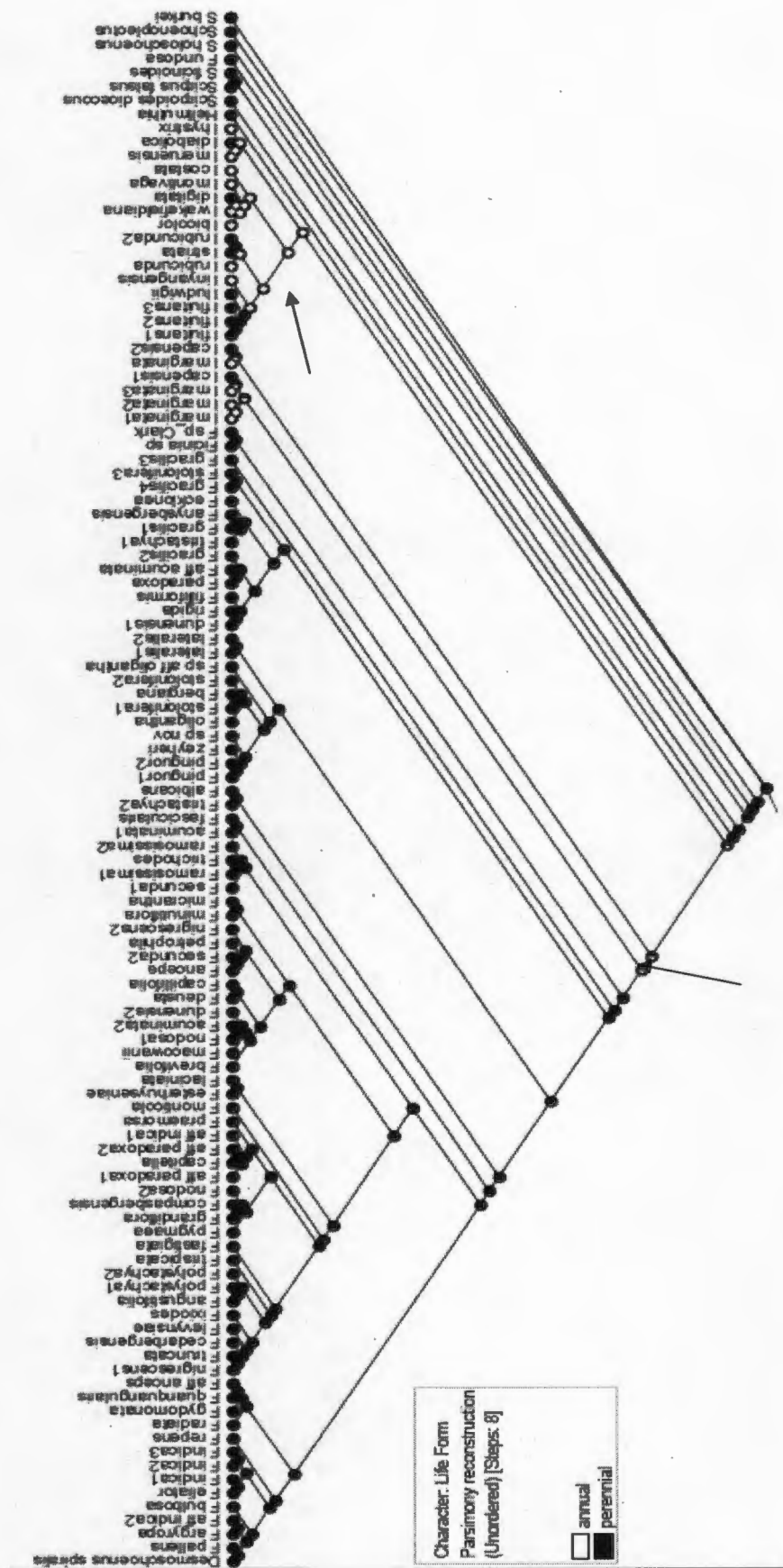


Figure 3a: Parsimony reconstruction of Life Form. The arrow indicates the ancestral state of the *Ficinia* ancestor and its closest relatives in the genus *Isolepis*. White represents annual habit and black represents perennial habit. The arrows indicates incidence of annual life form.

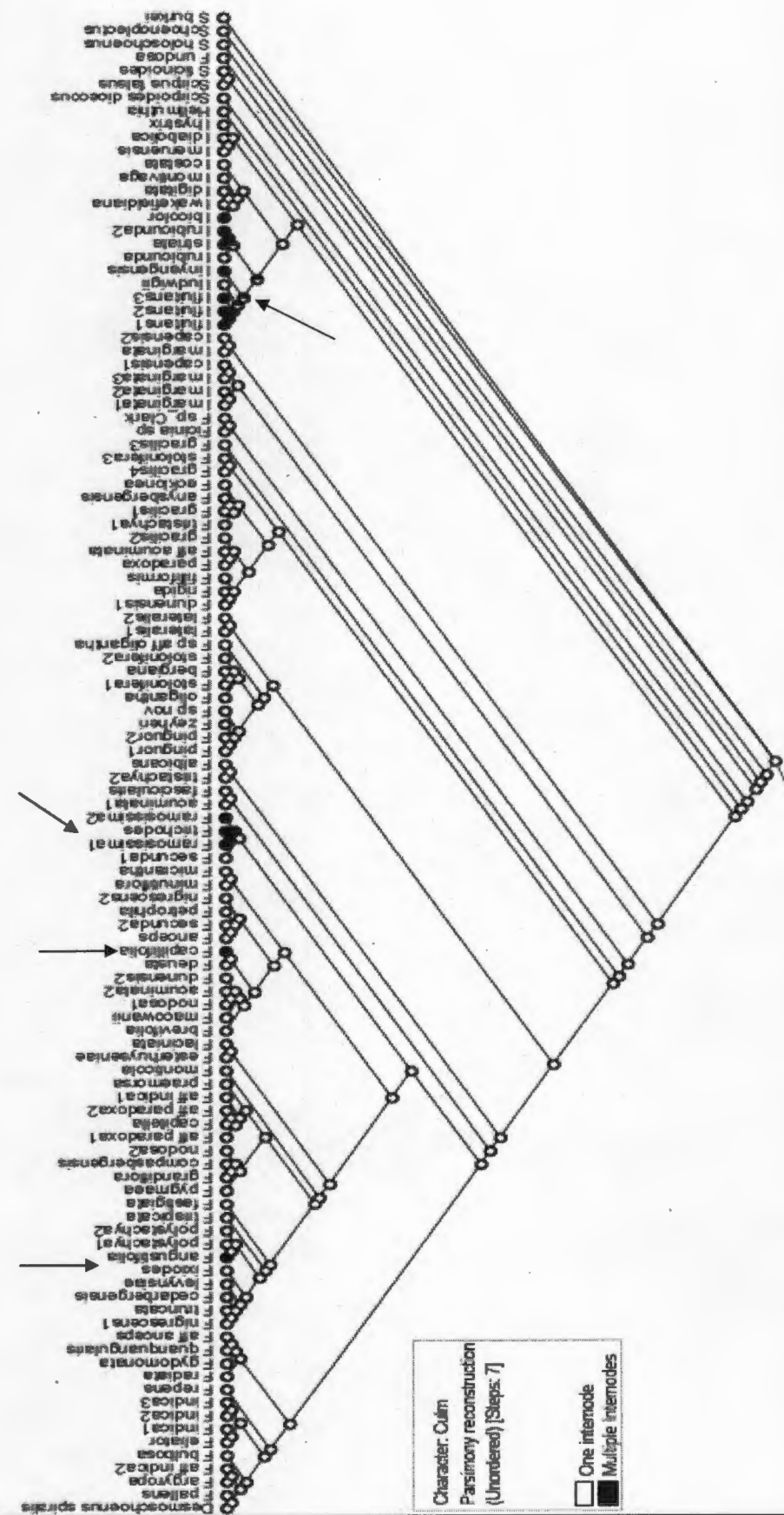


Figure 3c: Parsimony reconstruction of the number of internodes on the culm of the species. White indicates a single internode on the culm while black indicates multiple internodes. The arrows indicate the taxa in which multiple internodes have arisen.

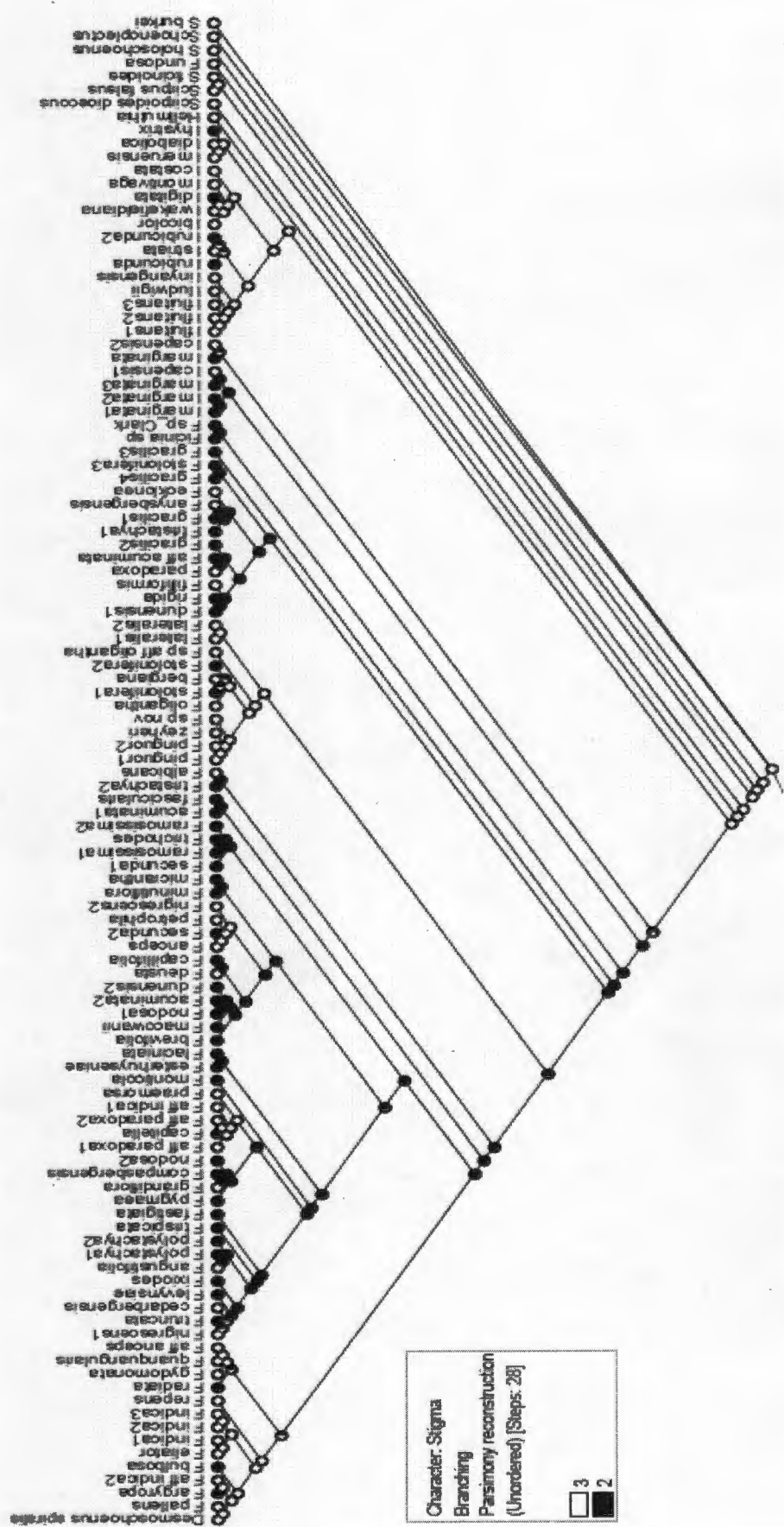


Figure 3j: Parsimony reconstruction of stigma branching around the nutlet. White indicates a state of 3 stigmas and black indicates a double stigma state.

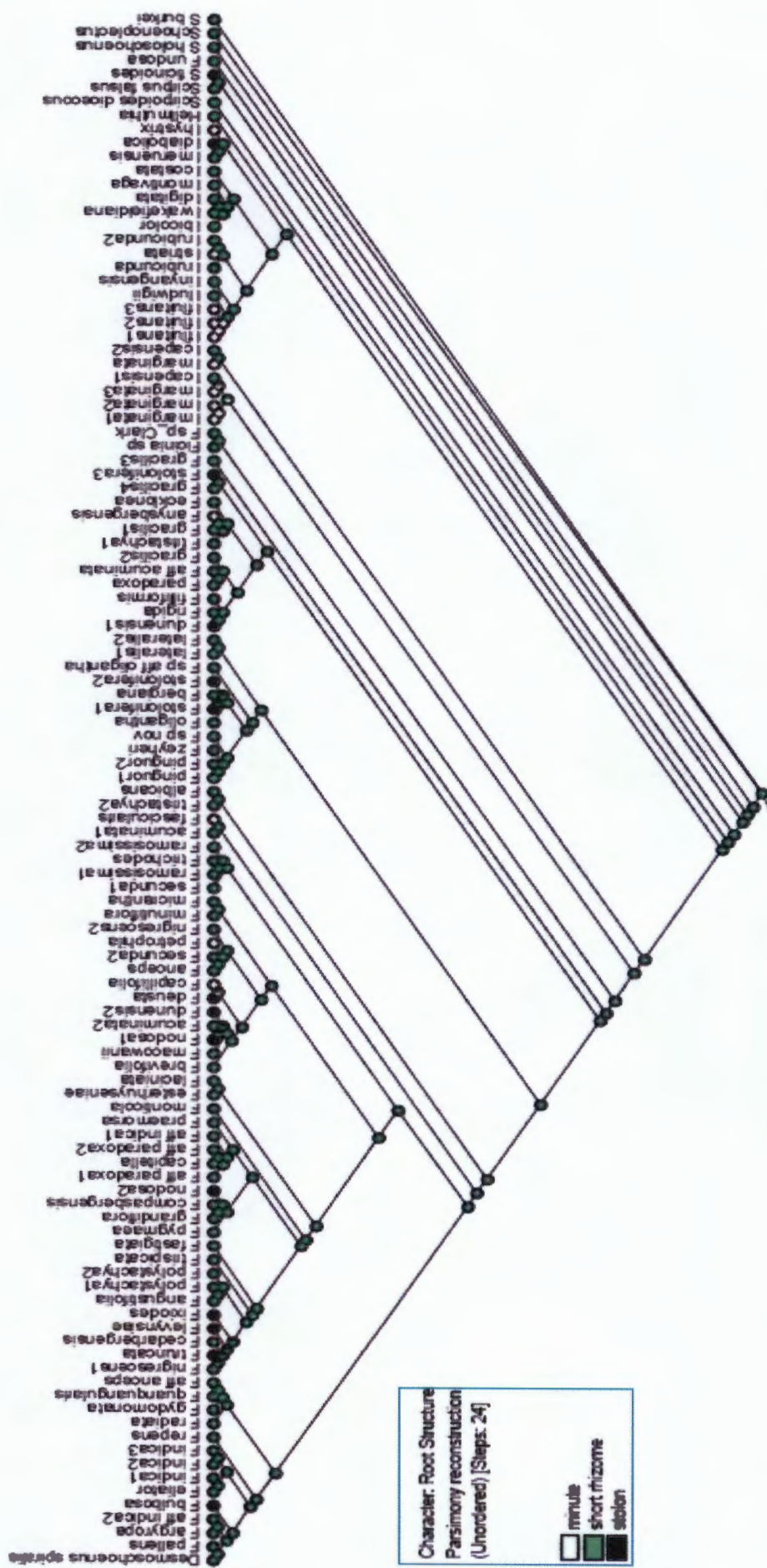


Figure 3m: Parsimony reconstruction of root structure in *Ficinia* and related genera. White indicates minute root structures; green indicates short rhizomes less than 5 mm from the subsequent culm and black indicates a stoloniferous root structure.

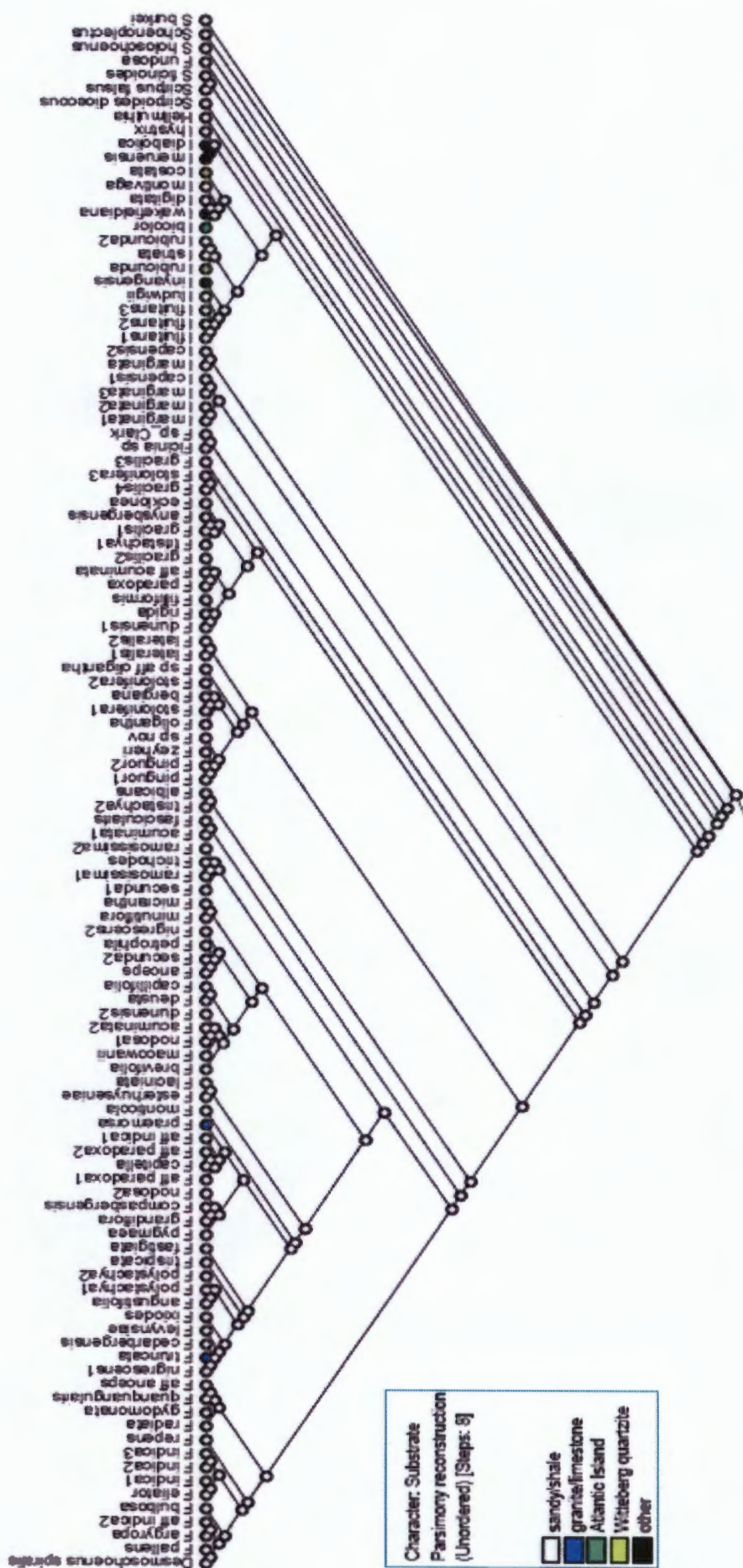
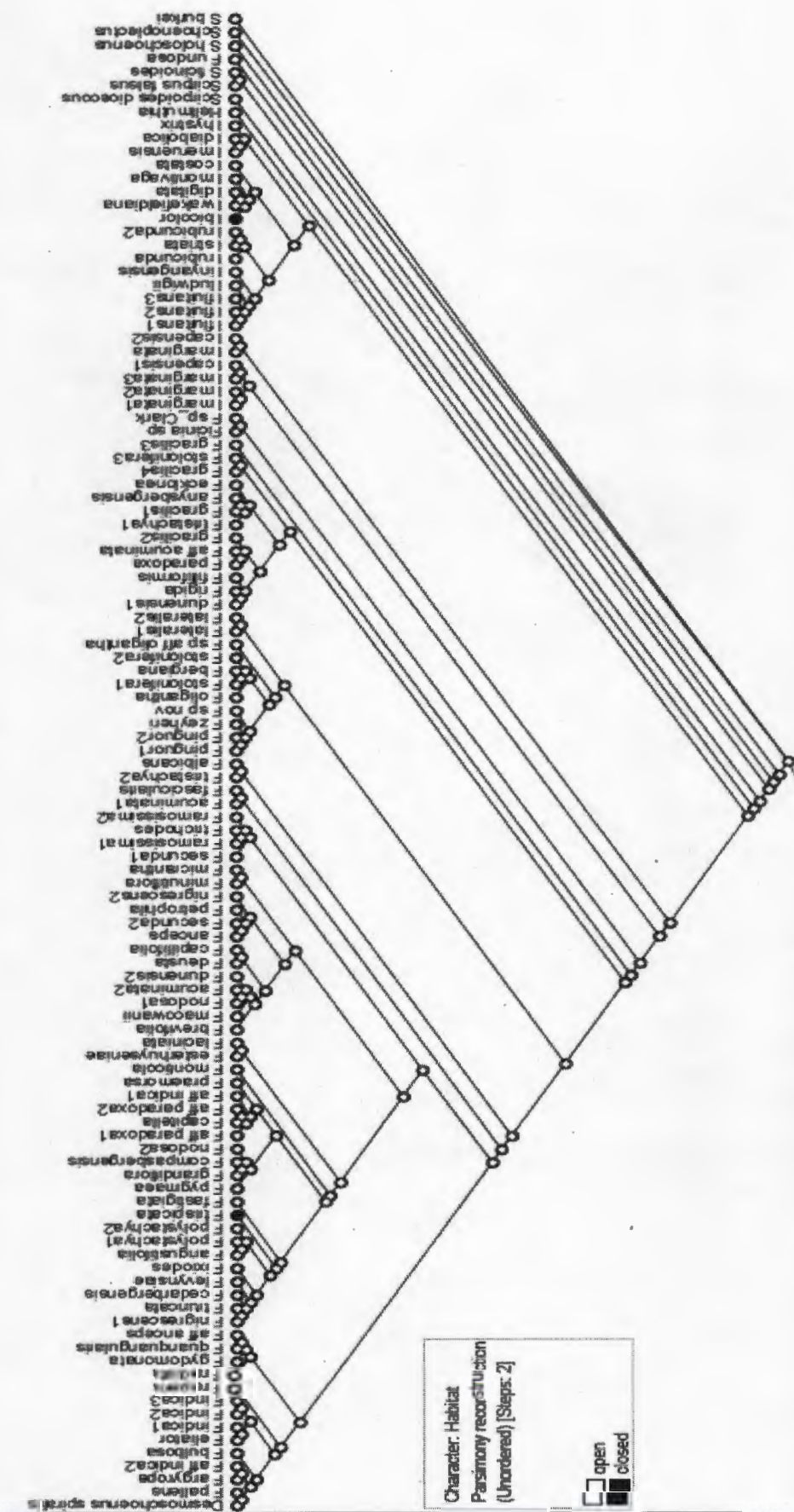


Figure 3n: Parsimony reconstruction of the substrate upon which species generally grow. White depicts the sandy/shale substrate of the South-Western Cape; blue depicts the granite and limestone outcrops; yellow depicts the Witteberg quartzite; green the volcanic Atlantic Islands and black depicts substrate of unknown origin.



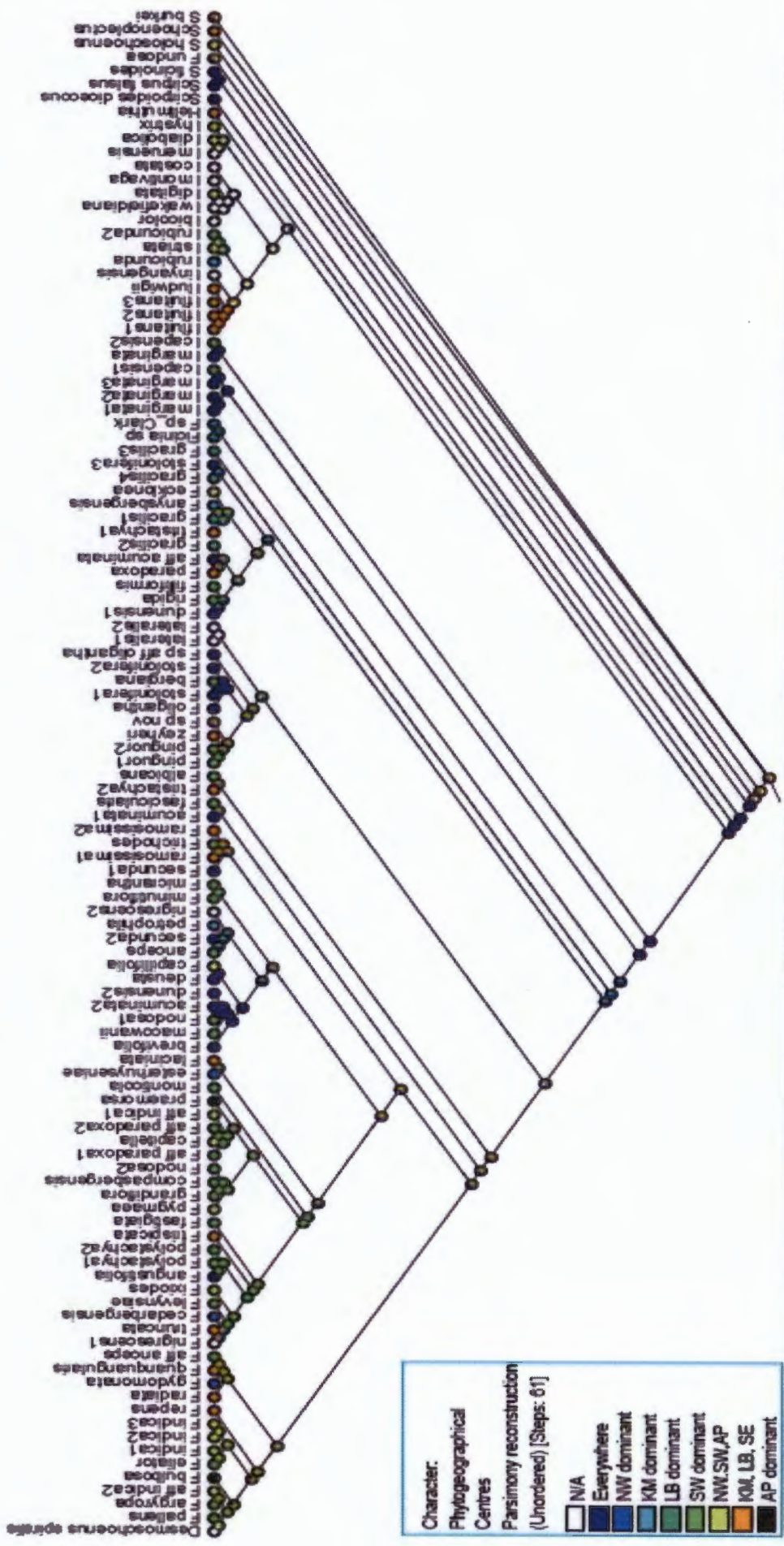


Figure 3p: Parsimony reconstruction of the major Phylogeographical Centres in the CFR. Centres are depicted as dominant or shared depending on the distribution of the species within the CFR. White circles in the tree represent the species that are absent from the CFR.

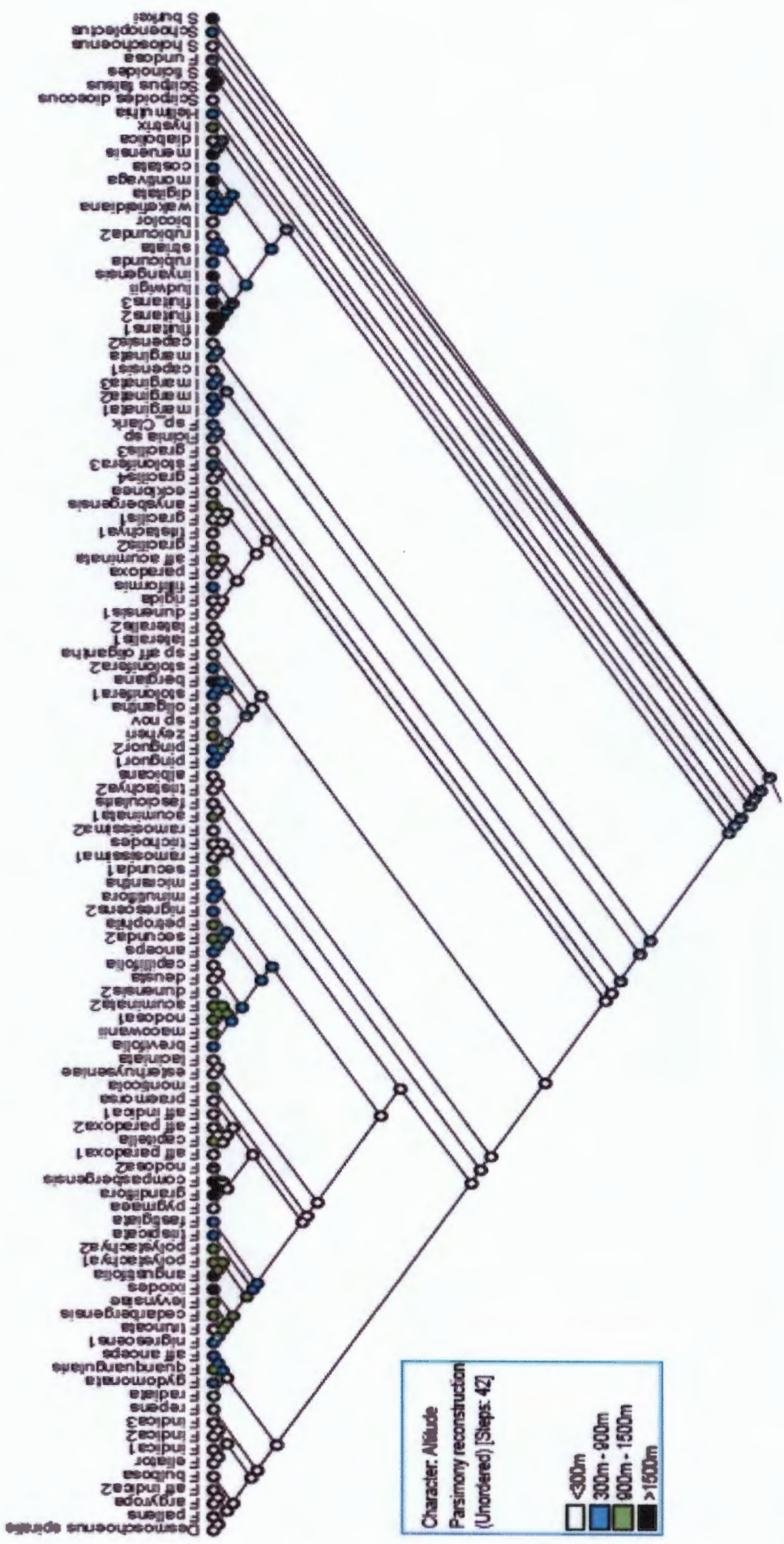


Figure 3q: Parsimony reconstruction of Altitude at which specimens are located. Categories are defined according to Goldblatt & Manning (2000) standards with intermediate intervals of 600m. White circles are indicative of altitudes below 300m i.e. lowland fynbos vegetation.

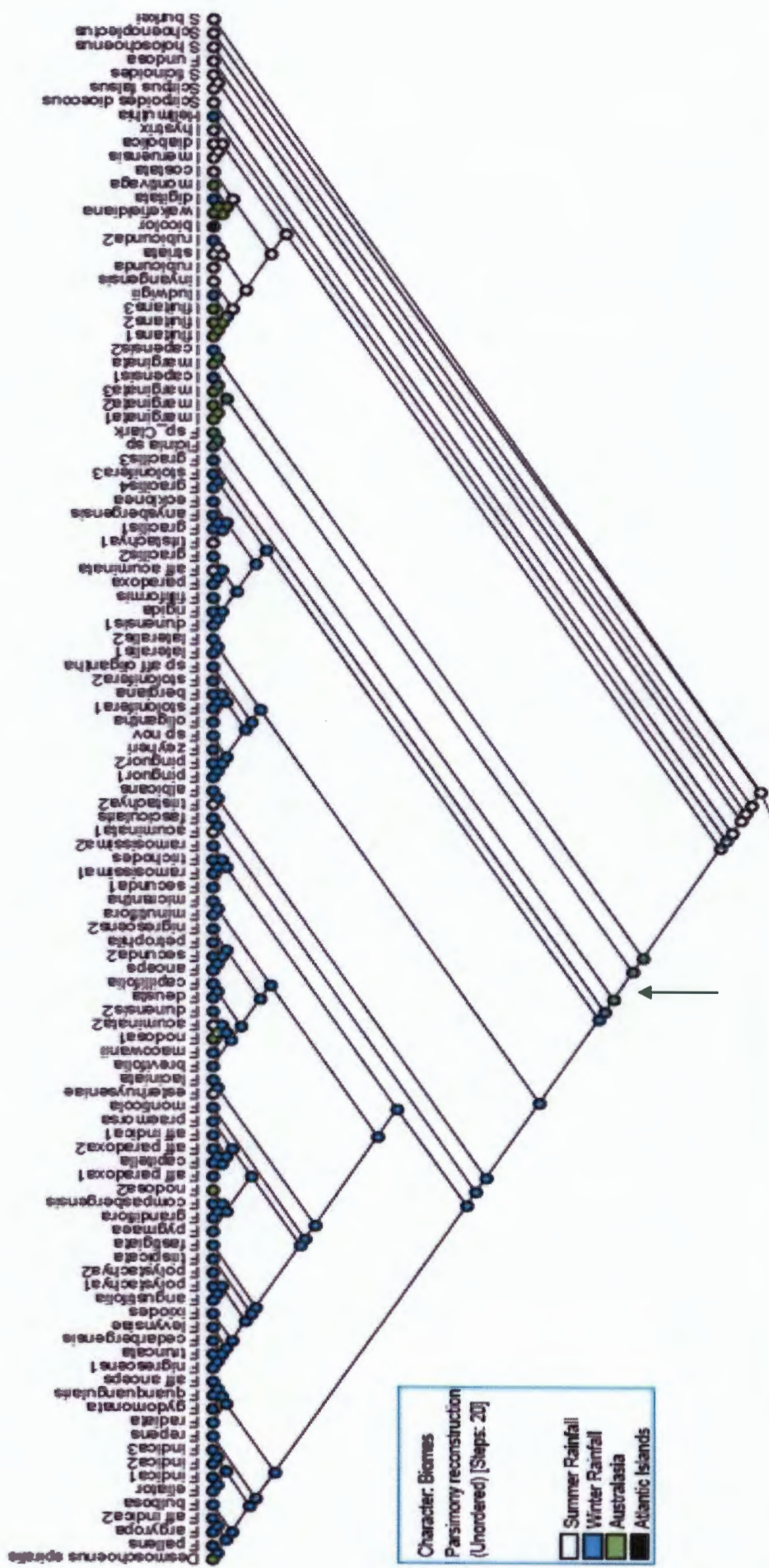
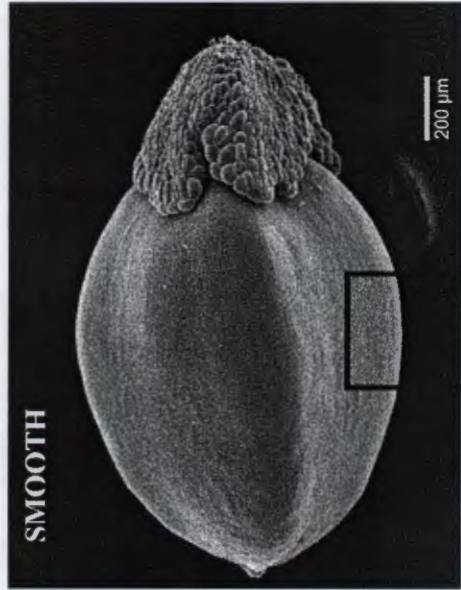
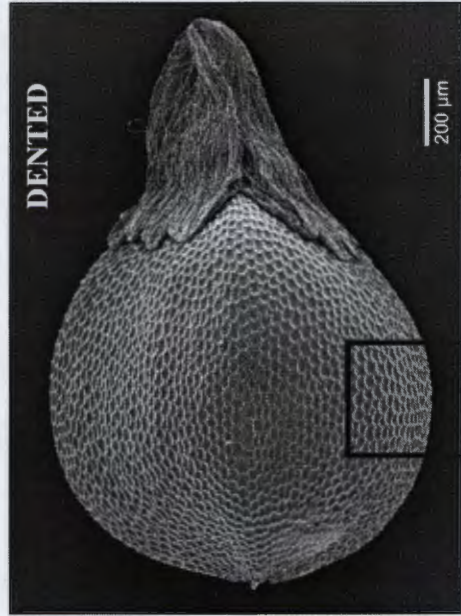


Figure 3r: Parsimony reconstruction for the character of Biomes inhabited. The arrow indicates the ancestral trait within *Ficinia* having the ability to inhabit both summer and winter rainfall regions but a greater propensity for winter rainfall regions.

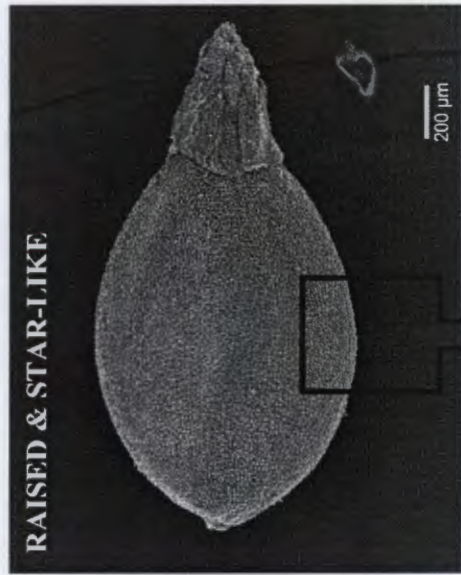
F. macowanii



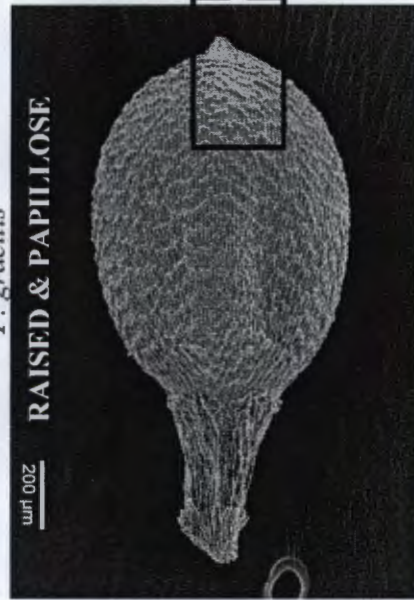
F. argyropa



F. oligantha



F. gracilis



F. deusta

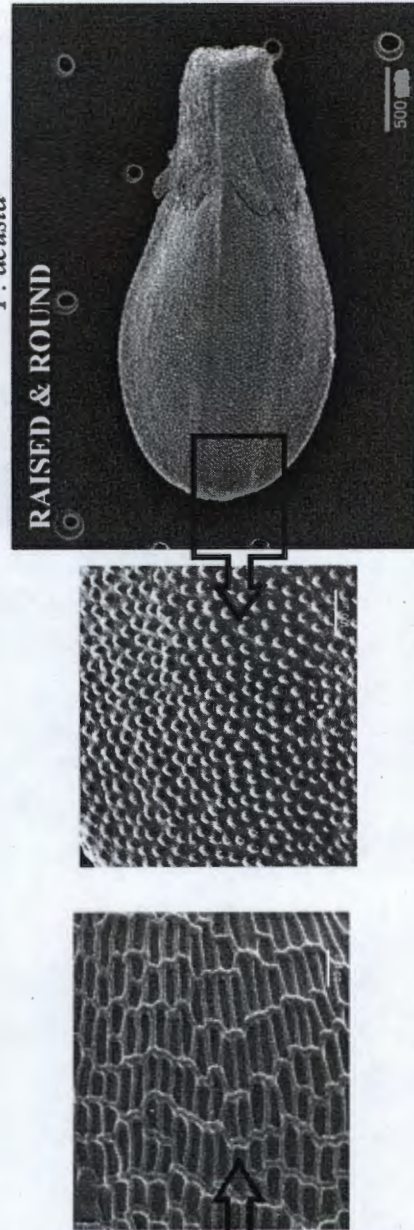


Figure 4: SEM photographs of the range in Nutlet Surface Morphology possible in *Ficinia* (Images Courtesy of Dr AM Muasya)

state and is retained among the majority of species studied (Figure 3h); the distichous state is derived in only a handful of species (e.g. *F. angustifolia*, *F. capillifolia*). The reconstruction of the gynophore disk in this study (Figure 3i) shows a single incidence of gynophore absence in *F. filliformis*. The ancestral state for stigma branching is one of 3 stigmas with the *F. pinguior*-*F. lateralis* and *F. pallens*-*F. repens* clades, for example, displaying a shift toward two stigmas (Figure 3j). Seed surface ornamentation reconstruction in Figure 3k has a number of features that bears closer inspection. The first is that the raised state for surface morphology one exhibited by the ancestor of the *Ficinia*, but the exact shape is not known. Furthermore, the species *F. gracilis* holds within in a large variation in nutlet surface morphology highlighting the existence of a known species complex. The spicate inflorescence type is evidently a derived character for the *Ficinia* and the sister genus *Isolepis* where the ancestral inflorescence is capitate (Figure 3l). While, in root structure reconstruction we see that having a short rhizome is not only an ancestral trait, but this state is for the most part common with only a handful (e.g. *F. dunensis*, *F. stolonifera* and *F. levynsiae*) of stoloniferous root structures evolving in *Ficinia* (Figure 3m). Shifts to alternate, non-sandstone derived substrates occurred only twice with in the *Ficinia* with two independent movements onto limestone (Figure 3n) by *F. praemorsa* and *F. truncata*. Similarly, inhabiting closed, forested habitats is a derived trait (in *F. trispicata*) with the majority of the species within the *Ficinia*-*Isolepis* clade inhabiting open areas such as fynbos veldt and rock overhangs (Figure 3o). The reconstruction of phylogeographic centre for the genus is largely biased by the presence of a few taxa in all centres (e.g. *F. oligantha*, *F. esterhuyseniae*, *F. stolonifera* and *F. deusta*). The extant taxa that show a marked preference for SW dominant or allied Phytogeographical centres (Figure 3p). There are, however, a number of taxa that are found exclusively in the eastern half of the CFR in the Karoo Mountains and Langeberg area. Within *Ficinia* in particular the ancestor seems to be a lowland plant occurring below 300m and directional movement in altitude is evident in the extant taxa (Figure 3q). The majority of *Ficinia* are winter rainfall region inhabitants (Figure 3r) with some *F. gracilis* having been collected in Kwa-Zulu Natal – a summer rainfall biome. *F. nodosa* occurs in the CFR, but it is also found in Australasia.

Discussion

Phylogenetic Relationships

Muasya *et al* (2009) inferred phylogenetic relationships within *Cyperaceae* based upon four (4) plastid DNA regions (*rbcL* gene, *rps16* intron, *trnL* intron and *trnL-F* intergenic spacer) using a parsimony analysis. Two distinct clades were identified – *Ficinia* and *Cyperus* – however; the relationships within the genus *Ficinia* within the *Ficinia* clade were poorly resolved. This study infers the relationships within the genus *Ficinia* by including morphological characters as well as nuclear DNA to two of the previous analysed plastid regions (*rps16* intron and *trnL-F* intergenic spacer).

Five (5) distinct clades formed within the *Ficinia* in the phylogeny represented in Figure 1. The relationships formed within these clades are broadly similar to those produced by Muasya (unpublished results). A number of placements in the phylogeny produced in this study bear closer inspection. The strong sister relationship resolved between *Isolepis* and *Ficinia* is expected with a number of earlier studies resolving this relationship as well (e.g. Muasya *et al* 2009, Dlodlu 2007, Muasya *et al* 2005). *Isolepis marginata*, an annual was resolved as more closely related to the *Ficinia* based on both purely molecular (not included in this study) and combined phylogenies. This generic overlap has been resolved in two other studies (Muasya & Simpson 2002 and Muasya *et al* 2009) as well. The presence of a gynophore disk at the base of its nutlet in addition to its phylogenetic position sister to *Ficinia* are strong pieces of evidence to suggest a reclassification of this species into *Ficinia* despite its annual life form. *Isolepis capensis* is also resolved along with *Isolepis marginata* as closer to the *Ficinia* than to *Isolepis*. This relationship needs further investigation, as *I. capensis* does not bear a nutlet.

again, 18 Sept 13
paraphyletic
with respect
to *Ficinia*
not sister

In this study, *Ficinia* is not monophyletic but rather paraphyletic as *F. undosa* is resolved as more closely related to the representatives of the genus *Scirpoides* than its own genus or even that of *Isolepis* (Figure 1 and 2). Several factors may play a role in this unusual placement. *Ficinia undosa* exhibits a large number of insertion-deletion events evident in the sequences of this species do make it significantly different from the other members of its genus. While the support for the relationship is low, it raises questions whether *F. undosa* is really a *Ficinia* as it lacks a gynophore and shares gross morphology with *Scirpus falsus*/S. *ficiniodes*. On the other hand, it lacks

perianth bristles, which characterise *S. falsus* and *S. ficinioides*. Generic position of this taxon therefore requires to be verified by further study.

Character Evolution and homology

An earlier study by Muasya *et al* (2009) reconstructed broad character homology within the family with two distinct clades identified by plastid DNA regions – the *Cyperus* and *Ficinia* clades. The character descriptions were broad especially in regard to *Ficinia* as a genus which was poorly represented in that study. The current study found the traits in the *Ficinia* clade as identified by Muasya *et al* (2009) were in large representative of the genus itself.

Life Form and Root Structure

The *Ficinia* exhibit a plesiomorphic perennial life form which helps to distinguish them from their sister genus *Isolepis* who are annuals (Figure 3a). From the *Ficinia* Clade as identified by Muasya *et al* (2009) *Isolepis* are the only annuals in the clade and so annuality is a derived character useful in distinguishing *Isolepis* from the rest of the genera in the family in the CFR. Annual life form is uncommon in the CFR, contributing 10% of vascular plants (Goldblatt & Manning, 2000), and appears to have arisen multiple times in *Isolepis*. Annuals have minute roots as is seen in the *Isolepis* in this study whilst most of the perennial *Ficinia* have rhizomes and some with stolons (Figure 3m). Annuals persist for short periods and this makes investment in robust root structures less important in comparison with producing offspring – in this instance seeds. Perennials, on the other hand, persist through periods in which both water and nutrients are particularly limiting (e.g. fireless summers in the CFR) and so investment in structures capable, in particular, of extracting nutrients from the soil is important.

Plant Size

Ficinia have for the most part retained the small plant size exhibited by the putative ancestor as is reconstructed in Figure 3b. In the instances where a larger plant size has evolved – in particular in the clade consisting of *F. compasbergensis* and *F. truncata* – a reversal from this apomorphic larger size to the plesiomorphic smaller size of less than 30 cm has occurred. In the context of Cyperaceae, wide variation of plant sizes

are observed, ranging from under 1 cm to 5 m in *Cyperus papyrus* (Goetghebeur, 1998).

Internodes on the Culm and Leaf Blade Size

The propensity for more than one internode on the culm is synapomorphic trait shared by a subset of the *Isolepis* proper clade consisting of *Isolepis fluitans* and *I. rubicunda*, but within the genus *Ficinia* this trait is autapomorphic occurring 4 times in one clade and only 2 of the species (*F. trichodes* and *F. ramosissima*) are closely-related one another (Figure 3c). In addition to having multiple internodes, *F. angustifolia* also is the only species to exhibit a leaf blade size smaller than 5 mm. The ancestral trait of larger leaf blades is retained all the *Ficinia* as well as in the *Isolepis* and other sedge genera studied (Figure 3e). Neither of these features makes a particular good diagnostic feature of the genus and at best we are able to say that ancestor of *Ficinia* was similar to that of its contemporaries in other genera exhibiting one internode and leaf blades greater than 5mm.

Leaf Sheath appearance

This character has been suggested to be useful to distinguish *Ficinia* from other genera (ref). The reconstruction here shows that the papery nature of the leaf sheath is not evident in all the species (Figure 3D). It is an autapomorphic trait unique to *F. cedarbergensis*, *F. aff. paradoxa*, *F. paradoxa*, *F. ixiodes*, *F. deusta*, *F. stolonifera* and *F. filliformis*. The presence of a papery leaf sheath is therefore a fairly good diagnostic character for the genus. The ancestral state is a leaf sheath that is not papery in appearance. Within the CFR, papery sheaths are also observed in *Tetraria* and it is most likely arisen by convergent evolution within particular habitats.

Position of Inflorescence

Figure 3f shows pseudolateral inflorescence position to be the ancestral trait for the genus. For the clade of *F. pallens* to *F. ramosissima*, the evolution of terminal inflorescences is synapomorphic as indicated. In general, more taxa in the genus display the derived terminal state than the pseudolateral state. However, reversals to the ancestral state have occurred in several taxa such as *F. cedarbergensis*, *F. quanquangularis* and *F. secunda*. Terminal inflorescence position has arisen independently in *Isolepis* as well, but most of the species in this genus have retained

the pseudolateral position (Muasya & Simpson 2002). Overall the ancestral state for the *Ficinia* clade as identified by Muasya *et al* (2009) in *Cyperaceae* is one of pseudolateral inflorescence position.

Stigma Branching

Retention of the ancestral trait of 2-branched stigmas occurs in the *Ficinia* with the evolution of 3-branched stigmas arising independently several times within the genus (Figure 3j). The distinction between 2 and 3-branched stigmas was not always clear cut in the specimens (e.g. *F. capitella*) and this overlap has been recorded by previous studies in other genera in the *Cyperaceae* (for e.g. Muasya and Simpson 2002). An example of this is *Isolepis cernua* that has both 2- and 3-branched stigma (Muasya & Simpson, 2002).

Number of Spikelets per Inflorescence

The ancestral state is to have a large number of spikelets per inflorescence (ranging from 2 to 10) and this is retained in almost all the species of *Ficinia*. Some species however have increase the number of spikelets to more than 10 per inflorescence and in a select number of cases the opposite has happened species such as *F. micrantha* only a single spikelet makes up the inflorescence. This vast reduction however is an autapomorphic trait unlike the increased number of spikelets, which appears synapomorphic in origin. As a diagnostic feature the number of spikelets is not particularly distinctive as a number of *Isolepis* share this ancestral trait (Figure 3g).

Inflorescence type

A capitate inflorescence is the ancestral state in the *Ficinia* and indeed in the *Ficinia* clade of the tribe *Cypereae* as identified by Muasya *et al* (2009). Multiple instances (most autapomorphic) of evolution to the spicate state have occurred throughout *Ficinia* in particular. Within the group consisting of *F. polystachya* and *F. angustifolia* this trait appears synapomorphic having evolved in the ancestor and continuing to be retained in successive cladogenetic events. In addition, a single incident of reversal from the derived state to the ancestral state has occurred in *Ficinia* with a similar reversal within the *Isolepis* (Figure 3l).

Glume Arrangement

Previous studies have attributed a spiral glume arrangement to *Ficinia* (for e.g. Musaya *et al* 2009) and to a large extent that relationship is reconstructed here as well. Only a handful of taxa within *Ficinia* exhibit distichous glume arrangement which appears to be an autapomorphic character in both *Ficinia* and *Isolepis*. The ancestral state is one of spirally arranged glumes and retention of this character is spread across the sedges (Figure 3 h). Distichous glume arrangement has been used to diagnose *Cyperus sensu lato* and having spirally arranged glumes is a plesiomorphic state for the family as a whole (Musaya *et al* 2009).

Gynophore Disk

This is the key morphological character that distinguishes *Ficinia* from the other perennial genera in the family as many of the other share a gross morphology similar to this genus, especially *Scirpoides*. The gynophore is an often three-lobed structure developed from the hypogynous stalk that cups the base of the seed. Both size and shape of the gynophore varies (see Figure 4) and this variation has been suggested as a character for identifying different species. The gynophore is present in all recognized *Ficinia* species present in this study (Figure 3i) with the exception of *F. filliformis*, where it has most likely been lost secondarily. This reconstruction shows that the ancestral state for all the sedges in this genus is the absence of a gynophore making the evolution of this character a distinct feature of the genus. The great conundrum of the gynophore present in *Isolepis marginata* may well be resolved by absorbing the species into *Ficinia* on the basis of its sequence homology with that of the members of the *Ficinia*. The presence of a gynophore in *Desmoschoenus* was used to hypothesize close affinity to *Ficinia* (Browning & Gordon Gray, 1996), a position confirmed by the present phylogenetic studies.

The function of the gynophore is as yet unknown and Gordon-Gray (2008) raises a number of possibilities for this structure's purpose. One suggestion is that the gynophore serves as an initial food reservoir for the germling. The fynbos is a fire-driven ecosystem (Goldblatt and Manning 2002) and the gynophore in *Ficinia* may indeed provide the seedling with a food source in the initial stages of growth before root development.

Nutlet Surface Morphology

Wide variation is observed in nutlet surface ornamentation. Majority of the *Ficinia* species have raised and star-like projections on the surface, but this character has arisen independently in several clades (Figure 3K). This reconstruction reveals no distinct pattern of evolution in seed surface ornamentation. An ancestral form capable of producing a variety of surface morphologies gives rise to a more specialized state in the extant taxa. The *F. gracilis* species complex possesses several ornamentation types, and it is hypothesized populations in Kwa-Zulu Natal are more closely related to those in tropical Africa than populations the Western Cape (Gordon Gray 2008), a feature supported by surface ornamentation data (Figure 3k). It should be noted however that within *Ficinia* itself having a raised and star-like nutlet surface morphology is more common in the extant taxa than any other form.

Substrate and Habitat

Existence in sandy, nutrient-poor open habitats defines the majority of the species in this genus. These traits are symplesiomorphic with derivations happening very rarely and almost always being a case of singular autapomorphic character evolution (Figure 3n and o). The movement of *F. praemorsa* and *F. truncata* onto limestone substrates independently are the only derivations in substrate within *Ficinia*. A shift in substrate may well have lead to the evolution of these two taxa into the distinct species present today via parapatric speciation. This hypothesis is supported by Goldblatt & Manning (2002) who identified movements onto clay, granite and limestone soils as triggers for local speciation events in various genera.

Phytogeographical Centres

The strong tendency toward the SW centre is apparent for the ancestor of *Ficinia* in the morphological reconstruction (Figure 3p). This tendency to a SW centre is retained in the extant taxa with a few instances of species in the western half of the CFR (defined as the NW, SW and AP centres) that contains the SW centre as well. The other 5 Phytogeographical Centres have only 1 or 2 species defined as restricted to these areas as opposed to more than 20 species with SW centre restricted distributions. The core diversity of the *Ficinia* in this study stems from the SW centre with several species able to inhabit all regions, but very few being restricted to the other 5 centres. The distribution of the *Cyperaceae* is difficult to analyse, but studies

show that the family may have developed after the Miocene (Muasya & Simpson 2002 citing Raven & Axelrod 1974).

Biomes

The biome that a species inhabits appears to be a good character for distinguishing the genus. The majority of the genus, with the exception of 3 summer rainfall species and the Australasian members such as *Desmoschoenus spiralis* (New Zealand endemic) and *F. nodosa*, is found in the winter rainfall biome (Figure 3r). *F. nodosa* is found both in South Africa and in Australia and this is a surprising distributional range within *Ficinia*. The ancestral state for the known species of *Ficinia* appears to be one of a winter rainfall biome inhabitant. Features of *Ficinia* that make it suitable for this biome are relatively shallow root systems (<10cm) and a flowering time that for most species incorporates the winter rainfall period in the CFR (Goldblatt and Manning 2000).

This genus is one of the largely sub-Saharan genera within the family Cyperaceae. Its sister genus, *Isolepis* R. Br. is older (around 18 million years – GA Verboom unpublished results). The genus is relatively young; its split from *Isolepis* reported at 14.1 million years ago (GA Verboom unpublished results). Yet, a large number of the species in this genus formed in the last ± 5 million years around the same time that the CFR experienced a shift toward the Mediterranean climate that now characterizes this region (GA Verboom unpublished results, Goldblatt & Manning 2002). This shift however, was not as catastrophic for the plant species of the CFR similar events in the other five Mediterranean floras of the world (Goldblatt and Manning 2002). In addition to this “stable-climate and physical heterogeneity” hypothesis for the extensive diversification of plants within the CFR and so also *Ficinia* the relationship between nutrient poor soils (a feature of the CFR noted by many studies) and high beta diversity (species turnover along a habitat or environmental gradient) must also be considered (Goldblatt and Manning 2002).

Altitude

In the *Ficinia*, inhabiting areas below 300 m is an ancestral trait and has been retained in most of the terminal species. Movement to higher altitudes have been derived in a number of groups within the genus, with either further increases in altitude (from 300m – 900m to 900m – 1500m) evident in the terminal or reversals back to the lower

altitudes. Altitudes intermediate in height appears more common than movement to extreme altitudes within this apomorphic trait (Figure 3q). While the climate did vary in the Pliocene-Pleistocene, the changes did not result in whole scale movement to higher altitudes for members of the *Ficinia* and this seems to support the notion of Goldblatt and Manning (2002) that the Cape Region was not as drastically impacted as its northern climatic counterparts.

Conclusion

The genus *Ficinia* Schrader is a sub-Saharan genus that has its centre of diversity in the Cape Floristic Region. This study has found the genus to be paraphyletic with a single species *F. undosa* resolved as sister to the *Scirpoides* outside of the limits of its own genus and that of *Isolepis* its sister genus. *Scirpoides* shares the gross morphology of *Ficinia*; the numerous insertion and deletion events evident in the sequences and the absence of a gynophore in *F. undosa* make its placement within *Ficinia* questionable. Further study into this species is warranted on this basis alone. The relationship between *Isolepis marginata* and the *Ficinia* resolved in this phylogeny has been reproduced in numerous other studies on this clade within the *Cyperaceae* and aside from its annual life form this species shares morphological and molecular homology with *Ficinia*, most notably the presence of a gynophore. Speciation in the genus is a topic that warrants further investigation.

While three (3) DNA regions as well as a number of morphological characters have been used in this study the inclusion of addition DNA regions especially those of the nuclear genome would greatly improve the resolution within the *Ficinia*. Many of the relationships resolved in this study had low support and the inclusion of additional regions should lend greater credence to the relationships inferred in this study.

Acknowledgements

I would like to thank first and foremost my supervisor, DR AM Muasya for the specimens he provided, his guidance as well as his time and patience in the duration of this project. Also, I would like to thank Mr Meshack Dludlu for his help and tutelage in both the laboratory and analytical aspects of this project. His guidance, especially in the latter stages of the project, has been invaluable. Last but not least I would like to graciously thank the Systematics postgraduate students and lecturers of the Department of Botany UCT who were always willing to answer questions and the National Research Foundation for the generous contributions that made this study possible.

Huelsenbeck JP & Ronquist F. 2003. Mr Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**(12): 1572 - 74

Huelsenbeck JP & Rannala B. 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology* **53**: 904 - 913

Kruger FJ & Taylor HC. 1980. Plant Species Diversity in Cape Fynbos: Gamma and Delta Diversity. *Vegetatio* **41**: 85 – 93

Linder HP and Hardy CR. 2004. Evolution of the Species Rich Cape Flora. *Philosophical Transactions: Biological Sciences*. **359** (1450) 1623 – 1632

Maddison WP & Maddison DR. 2009. Mesquite: A modular system for evolutionary analysis Version 2.71. <http://mesquiteproject.org>

Muasya AM. 2005. *Ficinia anysbergensis* and *F. esterhuyseniae* (Cyperaceae), two new species from the Cape Floristic Region of South Africa. *South African Journal of Botany* **71**(2): 197 – 200

Muasya AM & Simpson DA. 2002. A monograph of the genus *Isolepis* R.Br. (Cyperaceae) *Kew Bulletin* **57**: 257 – 362

Muasya AM, Simpson DA and Chase MW. 2001. A phylogeny of *Isolepis* (Cyperaceae) inferred using plastid *rbcL* and *trnL-F* sequence data. *Systematic Biology* **26**(2) 342 – 353

Muasya AM, Vrijdaghs A, Simpson DA, Chase MW, Goetghebeur P & Smets E. 2009. What is a genus in Cyperaceae: phylogeny, character homology assessment and generic circumscription. *Botanical Review* **75**: 52 – 66.

Muasya AM, Simpson DA & Goetghebeur P. 2000. New Combinations in *Trichophorum*, *Scirpoides* and *Ficinia* (Cyperaceae). *Novon* **10**: 132 – 133

References

- Davis JJ. 1995. A phylogenetic structure for the monocotyledons, as inferred from chloroplast DNA Restriction Site Variation, and a comparison of measures of Clade support. *Systematic Botany*. **20**: 503 – 527
- Dludlu, MN. 2007. Cyperaceae tribe Cypereae: phylogenetic relationships and evolutionary patterns of diagnostic characters. Honours Dissertation. University of Cape Town.
- Fitch EM. 1971. Towards defining the course of evolution: minimum change for a specified tree topology. *Systematic Zoology*. **20**: 406 – 416
- Gawel & Jarret. 1991. CTAB DNA Extraction Protocol. *Plant Molecular Biology Rep.* **9** 262 – 266
- Goetghebeur P. 1986. *Genera Cyperacearum*. Ghent: State University Ghent. 507 - 609
- Goldblatt P. 1978. An analysis of the Flora of Southern Africa: Its Characteristics, Relationships and Origins. *Annals of Missouri Botanical Garden*. **63**: 369 – 436
- Goldblatt P & Manning JC. 2000. *Cape Plants: A conspectus of the Cape Flora of South Africa*. Sterilitzia 9. National Botanical Institute.
- Goldblatt P & Manning JC. 2002. Plant Diversity of the Cape Region of Southern Africa. *Annals of Missouri Botanical Garden* **89**: 281 – 302
- Gordon-Gray KD. 1995. *Cyperaceae in Natal*. National Botanical Institute, Pretoria, South Africa.
- Gordon-Gray KC. 2008. Studies in Cyperaceae in southern Africa 41: The *Ficinia gracilis* Schrader complex – An Overview. *South African Journal of Botany* **74**: 167 – 171

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877 – 884

Ronquist F. 2004. Bayesian inference of character evolution. *Trends in Ecology and Evolution*. **19**(9): 475 – 481

Simpson DA & Inglis CA. 2001. Cyperaceae of economic, ethnobotanical and horticultural importance: a checklist. *KEW BULLETIN* **56**: 257 - 360

Swofford DL. 2002. *PAUP*: Analysis Using Parsimony, Version 4*. Sunderland, Massachusetts: Sinauer Associates

Taberlet P, Gielly L, Pautou G & bouvet J. 1991. Universal primers for the amplification of three non-coding regions of the chloroplast DNA. *Plant Molecular Biology* **17**: 1105 - 1109

Van der Niet T & Johnson SD. 2008. Patterns of plant speciation in the Cape floristic region. *Molecular Phylogenetics and Evolution*. Pp 1 – 9

Vridaghs A, Goetghegeur P, Muasya AM, Cairis P & Smets E. 2005. Floral ontogeny in *Ficinia* and *Isolepis* (Cyperaceae) with focus on the nature and origin of the Gynophore. *Annals of Botany* **96**: 1247 – 1264